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# Catecholamine - Derived Tetrahydroisoquinoline Formation in Rats During Alcohol Metabolism

Mostafa G. Bigdeli  
*Loyola University Chicago*

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CATECHOLAMINE - DERIVED TETRAHYDROISOQUINOLINE FORMATION  
IN RATS DURING ALCOHOL METABOLISM

by

Mostafa G. Bigdeli

A Dissertation Submitted to the Faculty of the Graduate School  
of Loyola University of Chicago in Partial Fulfillment  
of the Requirements for the Degree of  
Doctor of Philosophy

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1974

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## L I F E

Mostafa G. Bigdeli was born in Tehran, Iran. He received his doctorate degree in Pharmacy from Tehran University, Iran, in 1958. He completed a three-year course in Clinical Laboratory Science in the Medical School of Tehran University in 1961. He served as a chief pharmacist and later as a supervisor of a clinical laboratory in different hospitals in Iran.

After coming to the United States in August, 1968, he took a semester's courses in English and biochemistry at Northwestern University. Following training at Grant Hospital, of Chicago, he passed the National Registry Examination in Medical Technology in June, 1970. In September he joined the Department of Biochemistry and Biophysics of Loyola University for graduate study and received his M.S. degree from that Department in June, 1972, under the guidance of Dr. Michael Collins.

Mr. Bigdeli married Anvar Massoud of Isfahan, Iran, in 1967. They have a son Fathali, born January, 1973.

In addition to be a registered medical technologist by the American Society of Clinical pathologists, Mr. Bigdeli is a registered clinical chemist as well as a full member of American Association of Clinical Chemists.




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## PART ONE

### INTRODUCTION AND REVIEW OF THE LITERATURE

#### A. PURPOSE

In several recent biochemical theories of alcoholic dependence, the possibility that biogenic amines may play a role in the expression of withdrawal symptoms has been discussed (1,2). It has been suggested that acetaldehyde (AcD) formed from the metabolism of ethanol or catecholamine (CA)- derived aldehydes elevated due to AcD, condense with CAs in nerve terminals and chromaffin cells to produce active 1,2,3,4, - tetrahydroisoquinoline (TIQ) alkaloid agents.

To date there are no reports on the biosynthesis of TIQs in animal tissues during ethanol ingestion. The purpose of this research is to explore, with electron capture gas chromatography (EC/GC), the possibility of TIQ alkaloid formation in rat brain and in adrenal glands during acute or chronic intoxication with ethanol. The quantitative changes in CA levels during alcohol metabolism are also evaluated, using the highly sensitive chromatographic method.

#### B. BACKGROUND

There appears to be little doubt that alcohol markedly affects the function of the central nervous system (CNS), as reflected by euphoria, loss of motor control, unconsciousness and severe physiological dependence. Unfortunately, there is no one satisfactory explanation of the biochemical mechanisms by which ethanol induces its neurological effects. However, several lines of evidence connecting the biogenic amines with the depressive and addictive

actions of this drug have been put forth and are discussed in the following three sections.

#### I. EFFECT OF ETHANOL AND ACETALDEHYDE ON NEUROAMINE METABOLISM

Ample evidence indicates that the biogenic amines (serotonin or 5-hydroxy-tryptamine (5-HT), norepinephrine (NE) and dopamine (DA) are localized in specific neurons and nerve terminals in the CNS (3,4) and that they function as chemical transmitters (5). Interaction of these neuroregulatory compounds with the ethanol metabolite, AcD, might result in abnormal levels or types of amine metabolites which would underlie the behavioral aberrations manifested in clinical alcoholism (6). This relationship in fact has been the basis for the numerous studies seeking to elucidate biochemical changes involved in human alcoholism.

Reports of the changes in the pattern of urinary CA metabolites after the ingestion of ethanol at various dose levels have appeared frequently. Smith et al (7) were the first to show that ethanol altered the metabolism of infused NE in humans from the oxidative pathway (vanillyl mandelic acid or VMA formation) to the reductive pathway (3-methoxy-4-hydroxy phenylglycol or MHPG formation, Fig.1). Similar findings have been reported by Davis and co-workers (8) in that ethanol induced a shift from VMA to the glycol derivative after intravenous (i.v.) ethanol injection in humans. The alteration in urinary 5-HT metabolites after ingestion of ethanol has been reported to be similar to that in NE metabolism (9). The effect of ethanol on the peripheral metabolism of DA in

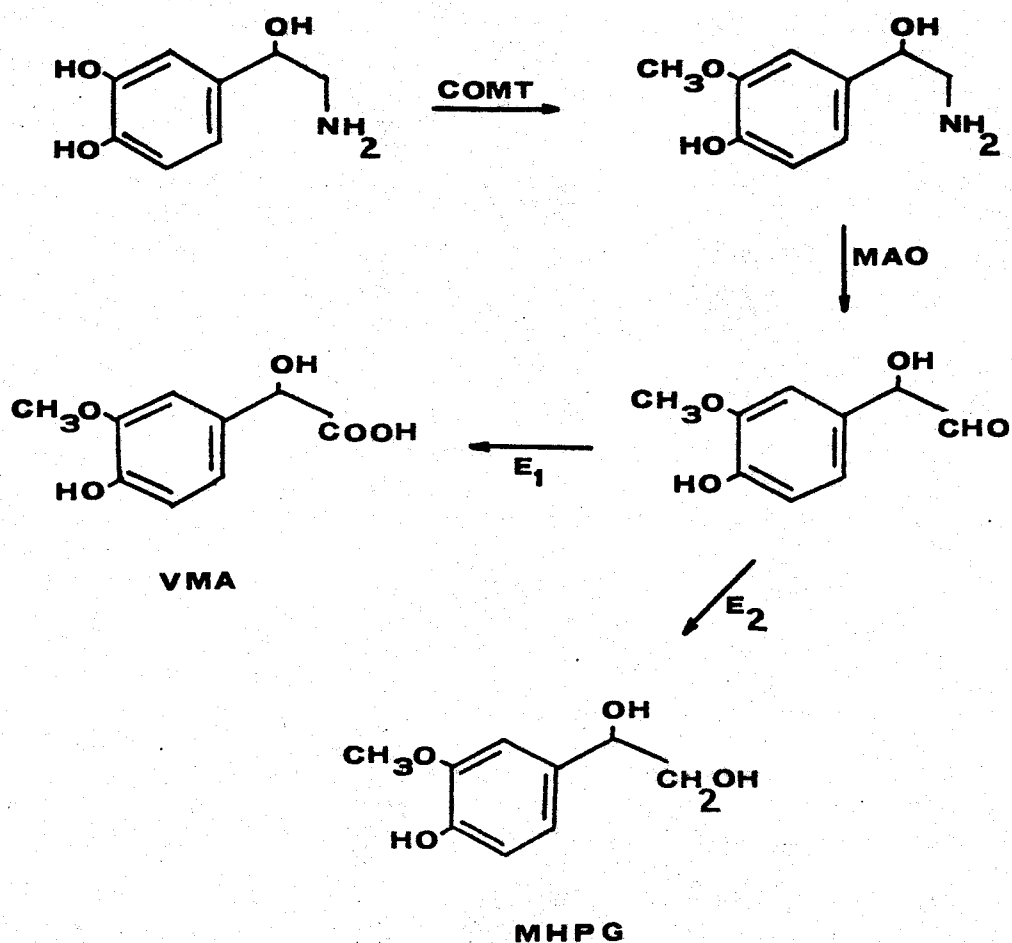


Fig. 1 Major pathway of norepinephrine catabolism in humans  
 E<sub>1</sub>, aldehyde dehydrogenase ; E<sub>2</sub>, aldehyde reductase.  
 Modified from A.A. Smith and S. Gitlow (7)

humans has not been studied. The results obtained in the ethanol-intoxicated rat (10) show a decreased level of acid metabolites of DA in urine, with no concomitant increase in the alcohol derivatives (3-methoxy-4-hydroxyphenyl ethanol and 3,4-dihydroxyphenyl ethanol).

Apparently, however, the shift to a reductive pathway does not occur in the CNS. Tytell and Myers (11) compared the effect of acute and chronic ethanol administration on  $^{14}\text{C}$ -5-HT metabolism in the rat brain. They did not observe large differences in CNS production of 5-hydroxy-tryptophol between the alcoholic groups and control. Instead, compared to control rats, they found a significant CNS conversion of  $^{14}\text{C}$ -5-HT to 5-hydroxyindole acetic acid (5-HIAA) in both acute and chronic ethanol treated rats, with a higher 5-HIAA level in acute ethanol treated rats. A similar observation of increase in 5-HIAA level in mouse brain, both after acute and chronic administration of ethanol, was reported by Tabakoff (12). The conflicting results reported on this subject are suggested to be due to the functional differences of 5-HT metabolism in the periphery and CNS (11). NE and DA have not been studied centrally with regard to the effect of ethanol metabolism.

Various explanations have been suggested for the effect of alcohol on peripheral CA metabolism: inhibition of monoamine oxidase (MAO) (13); a depletion of nicotinamide adenine dinucleotide (NAD, oxidized form, and NADH, reduced form) with a resultant increase of NADH/NAD ratio due to the oxidation of ethanol (8,12), and finally a competitive inhibition between AcD (derived from ethanol) and the intermediate biogenic aldehydes (MAO products) for



the active site on aldehyde dehydrogenase (8,14). Recently, supporting evidence for the competitive inhibition hypothesis has been reported (15,16). It was shown that AcD in the presence of excess NAD still caused a decrease in the formation of  $^{14}\text{C}$ -5-HIAA with a simultaneous increase in the neutral fraction (presumably 5-hydroxy-tryptophol) in rat brain homogenates (17).

## II. EFFECT OF ETHANOL AND ACETALDEHYDE ON NEUROAMINE STORAGE AND RELEASE PROCESSES.

Ethanol has been reported to increase urinary epinephrine (E) and NE levels in the rat (18). Klingman *et al* (19) reported that an acute sublethal dose of alcohol in dogs produced a marked increase in urinary E and NE. After adrenalectomy, only E excretion was decreased. These workers found that ethanol lowers the adrenal content of E without a significant effect on NE content. The main emphases of the above experimental works have been concerned with the action of ethanol on the storage and release of E from the adrenal medulla and depletion of NE from sympathetic adrenergic nerves (19).

In humans, ethanol consumption has been shown by several investigators to increase the release and excretion of CAs (20,21,22). Alcoholics who showed withdrawal symptoms in controlled intoxication studies continued to excrete larger amounts of urinary E and NE, whereas in those alcoholics not showing withdrawal symptoms the urinary CA concentrations returned to prealcohol baselines(21). This increase in urinary excretion of NE and E following cessation of ethanol intake in alcoholics during withdrawal appears to be

associated with a generalized activation of the sympathetic nervous system (22,23).

Some investigators very early suggested that the release of neuroamines by ethanol might well be due to its metabolites rather than, or in addition to, alcohol. Perman (24) found that i.v. injection of AcD in the cat produced a marked increase of E and NE levels in the suprarenal venous plasma. Therefore he suggested that AcD derived from ethanol may be the active agent responsible for the increased level of the urinary CAs. Using purified adrenal preparations, Akabane et al (25) were able to show that AcD intensifies the secretion of both E and NE from medulla. They indicated that CA release was closely correlated with an action of AcD on the membrane storage sites. Walsh and Truitt (26) reported that after i.v. administration of  $^3\text{H}$ -NE in cats and rabbits, AcD produced a much larger and more immediate release of the neuroamines into the plasma from peripheral nerve terminals than did ethanol.

CNS stores of biogenic amines also have been studied in regard to the effect of ethanol and in general the findings are controversial. Gursev et al (27) and Gursev and Olsen (28) found that i.v. administration of ethanol produced a significant reduction in rabbit brain stem 5-HT and NE. They felt that the effect of ethanol resembles that of reserpine in action, because they found that the reduction in central amine stores lasted several days. Other investigators have not found any effect of ethanol on the brain content of the amines (30,31,32,33). However, Corrodi et al (29) could demonstrate an alcohol-induced decrease in rat brain stem NE

in the absence of compensatory CA synthesis, by administration of  $\alpha$ -methyl-p-tyrosine, a tyrosine hydroxylase inhibitor.

Duritz and Truitt (34) suggested that this discrepancy may be because AcD is responsible for this effect. Intraperitoneal (i.p.) administration of AcD or of ethanol plus disulfiram induced a decrease in rat and rabbit brain NE, which was not seen with acute ethanol alone. They concluded that differences in brain amine liability to AcD, as well as the required time for maximal blood AcD levels after ethanol ingestion in different species, were critical factors.

### III. FORMATION OF ALKALOID CONDENSATION PRODUCTS FROM NEUROAMINES AND ALDEHYDES.

During ethanol metabolism the level of AcD, chemically a very reactive substance, increases in body fluid and cells. Table 1 demonstrates various biochemical substances which can easily react with AcD. A non-enzymatic condensation reaction has been shown to occur readily at physiological conditions between the amino groups of B-aryl ethylamines and carbonyl compounds, including AcD, to form varieties of cyclic compounds, B-carbolines in case of indolamines (tryptamine and 5-HT) and TIQs in case of m-hydroxylated phenethylamines (NE, E, DA).

#### a. CAs $\longrightarrow$ Simple TIQs (1-alkyl-TIQs)

In 1970, Cohen and Collins (1) reported that TIQ biosynthesis takes place in the medulla of isolated cow adrenal glands following perfusion with dilute AcD in isotonic phosphate buffer (pH 7) at 37°C. Later Cohen (44) confirmed TIQ formation in

TABLE 1

Known chemical reactions between acetaldehyde and biological substrates - Modified from M.J.Walsh(35)

Reactant	Product	Reference
A) Pyruvate	Acetoin	(36)
$\alpha$ - ketoglutarate	5-hydroxy-4-ketohexanoic acid	(37)
B) Coenzyme A (Co A)	Semi mercaptal formation; reported to decrease brain and liver levels of active Co A	(38)
C) Various amines	presumed Schiff base formation	
1. Indolamines	B-carbolines	(39)
5-methoxy-tryptamine	10-methoxy-harmalan	(40)
tryptamine and 5-HT	1-methyl-harmaline	(41)
2. Catecholamines (CA)	1,2,3,4-tetrahydroiso-quinolines (TIQs)	
epinephrine (E)	1,2-dimethyl-4,6,7-trihydroxy-TIQ (gigantinol*)( 1)	
norepinephrine (NE)	1-methyl 4,6,7, trihydroxy- TIQ	( 1)
dopamine (DA)	1-methyl-6,7-dihydroxy-TIQ (salsolinol)	(42)
D) Amino acid: DOPA	1-methyl-3-carboxy-6,7-dihydroxy-TIQ	(43)

\* This compound is hereafter referred to by its trivial name gigantinol, which is derived from the name ascribed to its 6,7-dimethoxylated isomer, the only isolated 4-hydroxy-TIQ, gigantine (from Carnegia Gigantea) (120).

the perfused adrenal gland at physiological (1-2 ug/ml) concentrations of  $^{14}\text{C}$ -AcD. The presumed reaction sequence of TIQ formation is presented in Fig.2.

Formaldehyde (HCHO)-derived TIQ biosynthesis was promoted in the rat adrenal glands during  $^{14}\text{C}$ -methanol metabolism (45). They observed the presence of the radioactive TIQs by thin layer chromatography, while E and NE were not radioactive. Further evidence for TIQ synthesis in vivo was obtained by Cohen and Barrett (46), who reported that TIQs were detected by fluorescence microscopy in lyophilized slices of adrenal tissue taken from rats chronically intoxicated with non-radioactive methanol.

Robbins (47) reported on the chemical reaction capability of several biogenic amines with AcD and suggested that such a reaction might occur in extracellular body fluid after ethanol ingestion. Yamanaka and coworkers (42) observed salsolinol formation in the homogenates of rat brain stem and liver incubated with  $^{14}\text{C}$ -DA and AcD. Based on these findings, Cohen and Collins (1) and Yamanaka et al (42) have suggested that physical dependency and withdrawal symptoms of alcoholics may be partly related to the formation of simple TIQs in nerve tissue.

b. CAs  $\longrightarrow$  Benzyloquinolines

Holtz and coworkers (48) originally described in vitro condensation of DA with its aldehyde, 3,4-dihydroxy phenylacetaldehyde, resulting in the formation of the 1-benzyl-TIQ, tetrahydropapaveroline (THP). Davis and Walsh (2) showed in vitro that AcD inhibits aldehyde dehydrogenase activity and leads to the accumula-

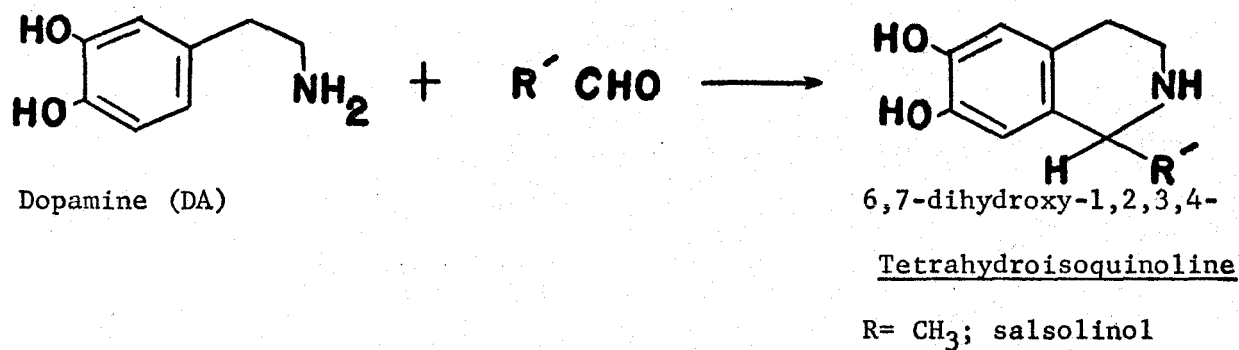
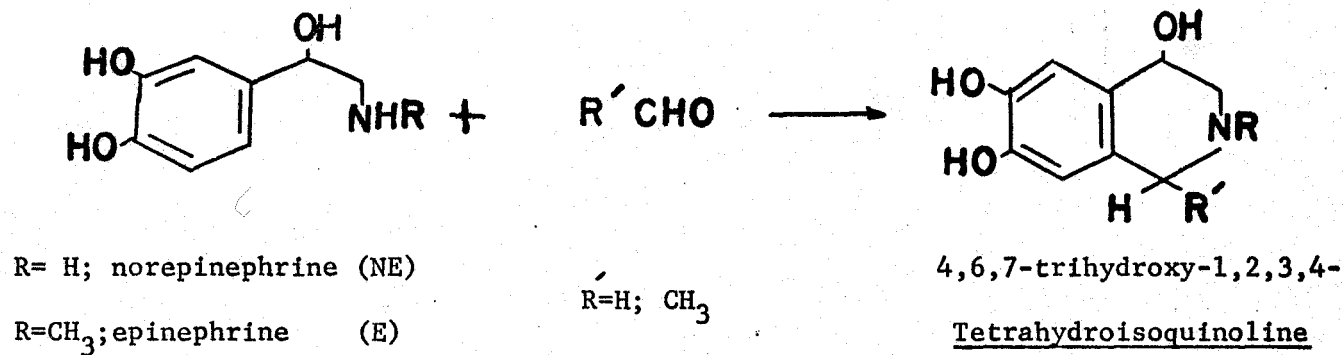


Fig. 2 Formation of tetrahydroisoquinoline (TIQ) alkaloids.

tion of 3,4-dihydroxy phenylacetaldehyde. This latter metabolite condenses with the second molecule of DA, and THP is formed ( Fig. 3). THP has been reported to be an intermediate compound in the biosynthesis of morphine (81). This had led Davis and Walsh to suggest (2) that alcohol addiction may be associated with the formation of this TIQ alkaloid. They reasoned that the formation of THP in brain tissue with relatively low aldehyde dehydrogenase activity is more likely than in other tissues which have high aldehyde oxidizing capacity.

It also has been stated that NE reacts with its biogenic glycol-aldehyde to form the analogous dihydroxylated THP (35). Similarly, a condensation product of 5-HT and its aldehyde was indicated by thin layer chromatographic (TLC) analysis. Thus, complex alkaloid formation appears to be a general reaction in vitro in the presence of MAO (35).

Sandler and coworkers (49) recently provided support for the hypotheses of simple (1) and complex (2) TIQ formation by detecting salsolinol and THP in the urine of Parkinsonian males who had been treated with therapeutic amounts of L-DOPA. Ethanol administration increased the level of urinary salsolinol in these patients.

#### c. Indolethylamines → B-carboline alkaloids

Taborsky and McIsaac (41) performed extensive experiments in an attempt to isolate pure B-carbolines from the reaction of a number of tryptamine derivatives with AcD. They were unsuccessful in isolating a single purified product from serotonin and AcD reaction. However, the impure products they obtained from this amine proved to be

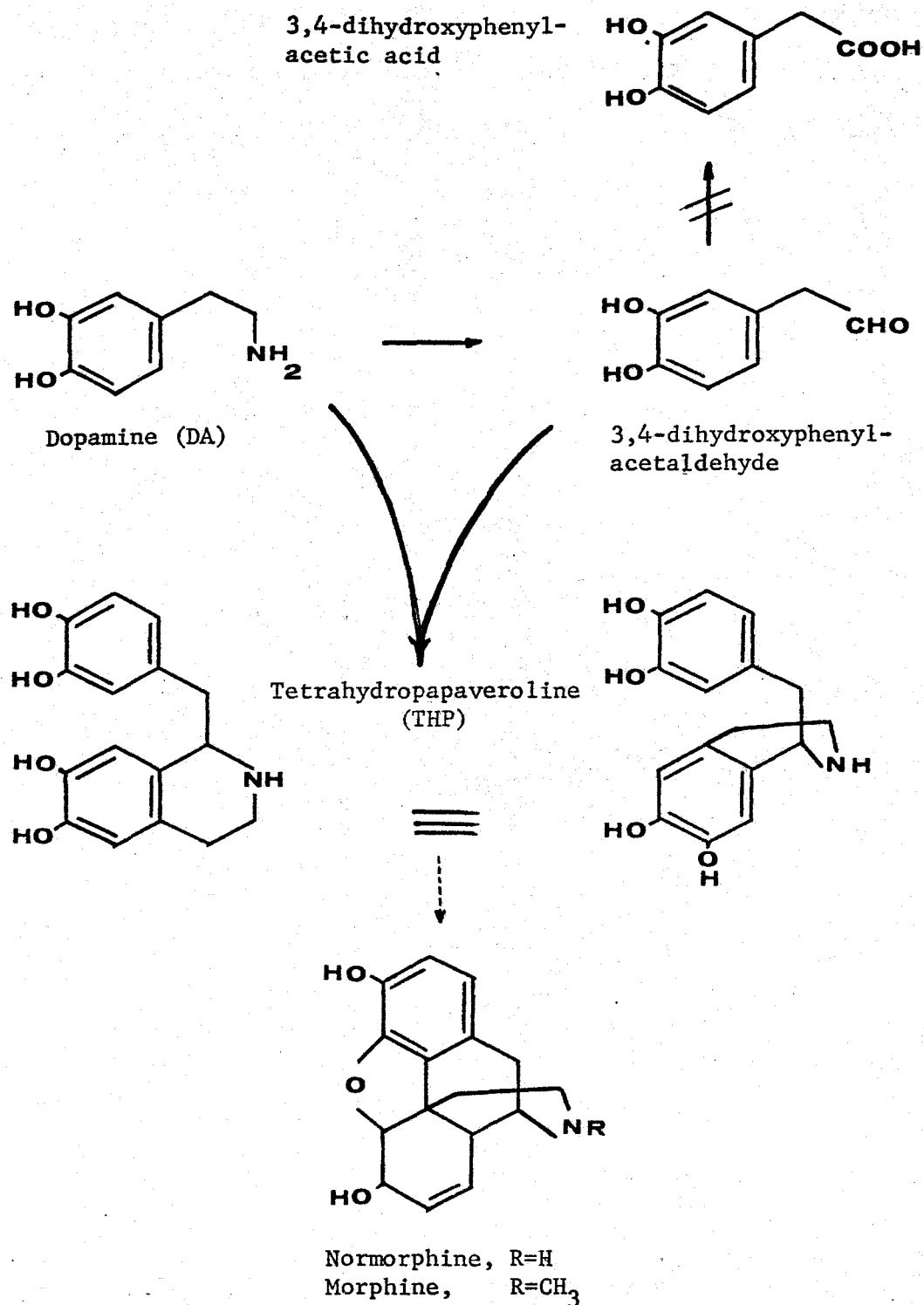


Fig. 3 Schematic representation of hypothesis illustrating an alteration in the metabolic disposition of DA produced by Ethanol ( $\nrightarrow$ ) with the postulated resultant formation of complex alkaloid derivatives.  
From V.E. Davis and M.J. Walsh (10).



potent MAO inhibitors and 5-HT antagonists and promoted aldosterone secretion. These are properties characteristic of B-carbolines. Later McIsaac (40) reported the isolation of small amounts of 1-methyl-6-methoxy-tetrahydro-B-carboline in the urine of rats treated with ethanol, 5-methoxy-tryptamine, iproniazid (MAO inhibitor) and disulfiram (aldehyde dehydrogenase inhibitor). This finding points out the possibility of a reaction such as the one depicted in Fig.4 to occur during alcohol ingestion in the pineal gland, a good source of 5-methoxytryptamine. Also very recently, Dajani and Saheb (50) isolated and identified 1-methyl-6-hydroxy-B-carboline from the urine of rats that were injected with ethanol and 5-hydroxy-tryptophan plus inhibitors of MAO and aldehyde dehydrogenase.

#### IV. CONDITIONS MODIFYING AMINE-ALDEHYDE CONDENSATIONS

The capacity of a reaction occurring between AcD and almost every amine has been demonstrated. Several manipulations could promote formation of TIQ alkaloids. Increasing amine or aldehyde levels are the two major factors. Amine levels may be increased by inhibiting MAO oxidation, as was done with indolamines (vide supra), or possibly by inhibition of catechol-O-methyltransferase (COMT) methylation. Inhibiting AcD metabolism is a way of enhancing this reactant's concentration. Therefore, by increasing the AcD levels as well as by elevating CA levels, theoretically the formation of TIQ alkaloids would be promoted. Since CA derived TIQs are good COMT substrates(51, 52), inhibition of O-methylation may favor the detection of catechol TIQs. In the case of THP formation, it presumably can be promoted by any drug that competitively inhibits aldehyde dehydrogenase in tissue.

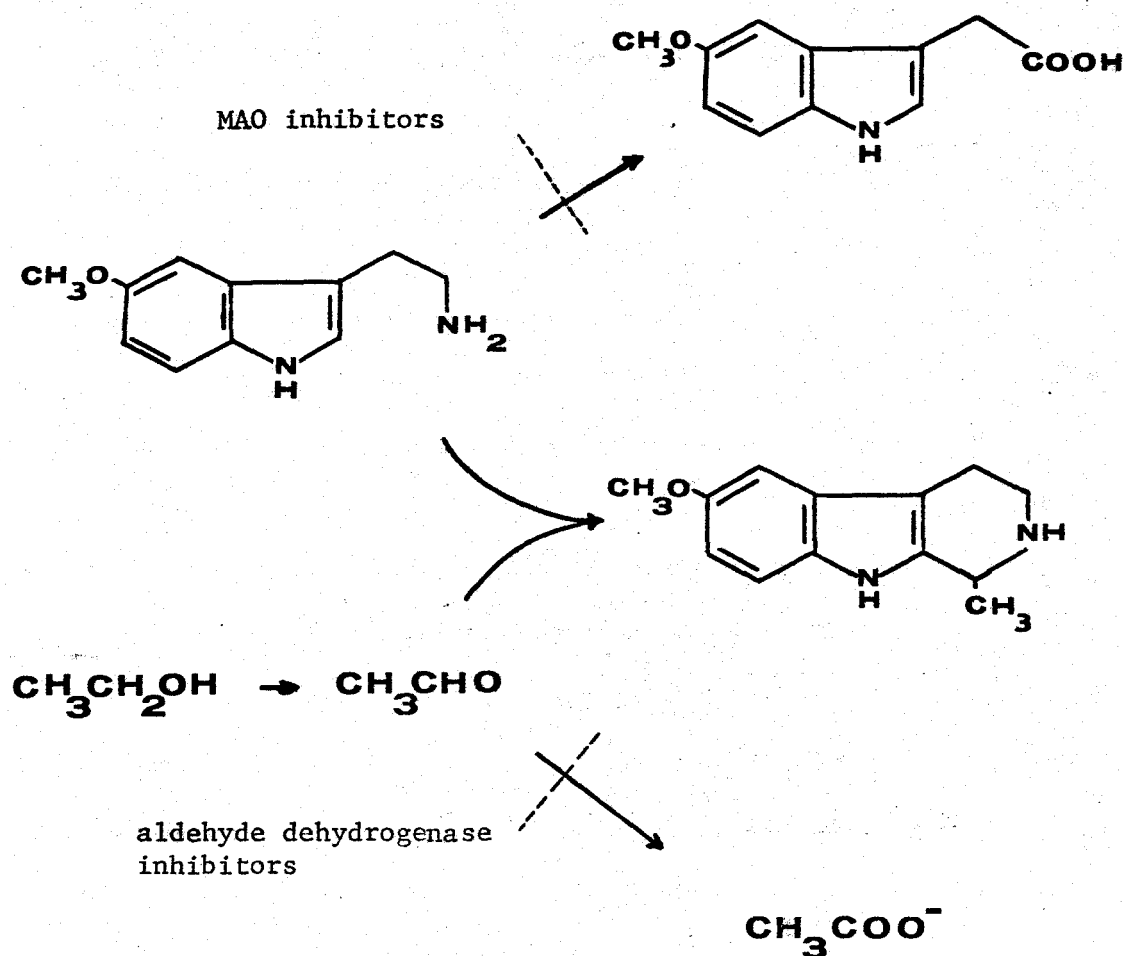


Fig. 4 Proposed pathway for the formation of B-carboline alkaloids from indolamines, modified from W.M. McIsaac (40)

## V. RELEVANT PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES ON TETRA-HYDROISOQUINOLINES

Early pharmacological research on simple TIQs has been discussed (53,62). More recently it has been noted that simple TIQs in vitro are accumulated by rat iris (54). This accumulation could be blocked by desmethylinipramine (an inhibitor of CA uptake). Similarly, TIQ uptake by central catecholaminergic nerve terminals and inhibition of CA uptake have been reported. Cohen et al (55) observed that accumulated radioactive CAs in rat brain homogenate were released by salsolinol incubation. Simple TIQs derived from E and NE, biosynthesized in AcD-perfused cells, could be released by sympathetic stimulation (56). These findings indicate that TIQ alkaloids share the same transport and/or storage sites in the nervous system and adrenal as the CAs. Therefore, Cohen (57) has suggested that TIQs might be capable of acting as "false" adrenergic transmitters. TIQs of the salsolinol type have been shown to have both central and peripheral pharmacological effects (58,59). These alkaloids may have either CNS depressant or stimulant and convulsant properties (58,59), lypolytic activity (60,61), and various effects on smooth muscles (59).

As discussed by Collins (63), very few simple alkaloids possessing a hydroxy group at the 4-position on the TIQ ring are known to occur naturally, and no physiological studies have been done on synthesized compounds analagous to those formed from the condensations of E and NE with AcD. Recently, however, some evidence has been obtained suggesting that 4-hydroxy-TIQs may be key intermediates in the biosyntheses of several classes of complex alkaloids (64).

On the other hand, THP has been more fully investigated. Some of its pharmacological effects on respiration, blood pressure and smooth muscle were described in 1910 (65). This alkaloid is sympathomimetic (65), evoking positive inotropic and chronotropic responses and decreases in blood pressure with accompanying increases in peripheral blood flow (10). In addition, relaxation of isolated smooth muscle preparations such as uterus, intestine and trachea is produced by THP (66). Furthermore, mobilization of lipid both in vivo (67) and in vitro (68) is enhanced by this alkaloid. All of the pharmacological effects reported for THP can be prevented by B-adrenergic blocking agents (65,66,67,68).

#### VI. THE POSSIBLE INVOLVEMENT OF NEUROAMINE-DERIVED ALKALOIDS IN ALCOHOLISM.

Ethanol directly or indirectly affects neuroamine metabolism, but the significance of these effects in relation to physiological and behavioral aberrations in alcoholism has not been defined. However, there are several theories proposed to account for the above-mentioned abnormalities found in human alcoholism. Several groups of workers believe that the condensation products of biogenic amines and aldehydes may be important metabolites in the utilization of ethanol. The first general hypothesis related to this topic came from McIsaac (40). In 1961, he put forth a general biochemical concept of mental disease. He suggested that normally circulating AcD in the body may condense with 5-methoxy-tryptamine (O-methylated serotonin) to form 10-methoxy-harmalan, a potent serotonin antagonist and MAO inhibitor.

Cohen and Collins hypothesized (described in part I) that, due to the ease of biosynthesis of TIQ alkaloids in adrenal tissue during AcD perfusion, the possibility of TIQ formation at adrenergic fibers of the sympathetic nervous system and brain is likely (1). If so, the TIQ alkaloids, either actively secreted or leaked from nerve termini, could contribute to the behavioral changes observed in alcoholic people. They also suggested that the signs of physical dependence such as tremulousness, hallucinosis and seizures which occur when concentrations of alcohol in blood (and brain) are falling may be due to persistent physiological actions of TIQ alkaloids.

Amit and Stern (69) suggested that NE-derived TIQs were synthesized in the nervous systems of their experimental rats. The animals were made alcohol dependent by electrical stimulation of the lateral hypothalamus. Free choice of alcohol consumption by increasing the concentration of alcohol daily permitted the ingestion of significant volumes of alcohol. A reasonable model was proposed for the role of TIQ thought to be formed in the synaptic gaps by interaction of AcD with NE. Increased precursor NE levels were suggested to occur due to the electrical stimulation. NE-derived alkaloids would be taken up by the same presynaptic cells from which the NE was initially released. As a result, the presynaptic cells involved would adapt to this new TIQ substance and it gradually would become the neural transmitter for that part of the nervous system. Once adaptation to alkaloid is complete, the neural system presumably cannot function well without it. Thus, the system would require regular intake of alcohol in order to

function. Earlier investigators (70,71) had brought out the same sort of hypothesis suggesting that alcoholics may have an impaired sympathetic nervous system which may be normalized by ethanol.

Davis and Walsh (2) have put forth the possibility of THP formation in the CNS and its involvement in the disease of alcoholism, although this theory, like the simple TIQ hypothesis, is controversial (72,73). These workers demonstrated the augmentation of THP biosynthesis when ethanol or AcD and DA were incubated with brain or liver homogenate. Furthermore, it is known that in plants THP is a natural biosynthetic intermediate in the synthesis of morphine alkaloids (81).

It should be added that indirect support of addictive TIQ formation was reported by Sprince and coworkers (74). These investigators found that, compared with controls, rats on a diet supplemented with DOPA tend to prefer an ethanol solution to water.

## VII. ANIMAL MODELS OF ALCOHOLISM

Induction of alcohol addiction in laboratory animals, to provide a model to study the neurochemical aspects of alcoholism, is particularly important ( 137, 138 )

Numerous workers have attempted to produce signs of physical dependence and subsequent withdrawal syndromes with ethanol in a variety of animals. Very little success has been achieved in inducing ethanol withdrawal syndromes in animals by the voluntary drinking of aqueous ethanol solutions. In rodents, usually most ethanol is consumed during the night, leaving the daytime for recovery. But Freund (75) found that liquid diet in which 35% of

calories came from ethanol produced physical dependence in mice within four days. Ellis and Pick (76) developed an alcohol intubation method that produced human-like withdrawal symptoms in monkeys and dogs that could be suppressed by ethanol administration. Recently Majchrowicz (77) has reported alcohol dependence in rats after eight days of gastric intubation. Pieper et al (78) recently found that young chimpanzees voluntarily consuming ethanol demonstrated withdrawal symptoms similar to those observed in humans. Goldstein's method (79) of inducing physical dependence involves closed chambers in which mice breathe ethanol vapor.

#### VIII. GAS CHROMATOGRAPHIC DETERMINATION OF CATECHOLAMINES

In gas chromatographic (GC) analysis, it is often necessary to prepare derivatives of substances to be analyzed. The aim of derivative formation is to increase the volatility of the compound and to reduce its polarity. In addition, derivative formation may give information by causing changes in retention time (80,99).

GC analysis, potentially as sensitive as fluorometry, may be the most specific method available for the estimation of biological amines. With electron capture (EC) detectors, as little as  $10^{-12}$  grams (picogram) or less of some compounds can be detected, but this requires certain halogenated derivatives which are good electron acceptors.

GC analysis of CAs have been done by a number of groups using trimethyl-silyl derivatives. Horning et al (82) published a method of O, N-trimethylsilyl formation of CAs with trimethylsilyl imidazole and bis-trimethylsilyl acetamide. Fluoroacyl deriva-

tives of CAs for EC detection recently have been employed by the workers in the field. Recently Arnold and Ford (83) prepared different fluorinated derivatives of CAs using trifluoroacetic (TFA) anhydride, pentafluoropropionic (PFP) anhydride and heptafluorobutyric (HFB) anhydride in acetonitrile. HFB derivatives showed the highest sensitivity of the three. They measured CA levels in rat brain tissue. Martin and Ansell (84) reported EC/GC estimation of DA, NE, and 5-HT in rat brain using TFA-anhydride in acetonitrile. Koslow and coworkers (85), using mass fragmentography, a technique in which the mass spectrometer is used as a single or multiple ion chromatographic detector, assayed NE and DA in 0.1 mg tissue in the picomole range. They used PFP-anhydride in ethyl acetate as a derivatizing agent.

#### IX. GAS CHROMATOGRAPHIC DETERMINATION OF TETRAHYDROISQUINOLINE ALKALOIDS.

Only a limited number of TIQ studies have been employed using GC methods. Agurell (86) has carried out GC studies on TIQ plant alkaloids with a flame ionization (FI) detector. Cashaw et al (87) developed a flame ionization GC method for determination of TIQs including salsolinol and THP in urine. They have used trimethylsilyl derivatives which are not applicable for EC detectors. More over, this work was not applied to the study of endogenous amines in tissue. Sandler et al (49) performed EC-GC analyses of the urine from Parkinsonian patients for the detection of salsolinol and THP, employing PFP derivatives. This work was not concerned with the simultaneous separation and measurement of CAs and TIQs.



## PART TWO

### EXPERIMENTAL SECTION

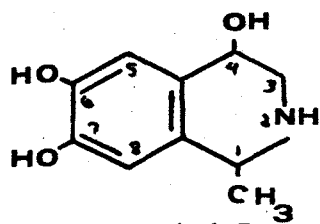
#### A. MATERIALS

##### I. CHEMICALS

Derivatizing agents (HFB anhydride, PFP anhydride, silylation grade acetonitrile) were purchased from Pierce Chemical Company. All of these reagents were kept refrigerated. Ethyl acetate (Pierce Chemical Company) was distilled before use. L-(-)-E and L-(-)-NE were obtained from Regis Chemical Company. The hydrochloride salts of metanephrine and normetanephrine were purchased from Winthrop Laboratories. Salsolinol hydrobromide was obtained from Aldrich Chemical Company. The hydrochloride salts of 4-hydroxy-TIQs were synthesized in our laboratory (88). The structures of those used in our experiments are shown in Fig. 5. THP was obtained as a gift from Welcome Laboratories, England. Aluminum oxide ( $\text{Al}_2\text{O}_3$ ) (Woelm, neutral activity grade) was activated by the procedure of Anton and Sayre (89) and was stored in a desiccator over drierite. Perchloric acid and sodium metabisulfite (J.T. Baker Chemical) were c.p. grade. Bitartrate salts of radioactive CAs ( $^{14}\text{C}$ -DA,  $^{14}\text{C}$ -E) were obtained from Amersham/Searle. All solvents for scintillation counting were purchased from Beckman Chemicals.

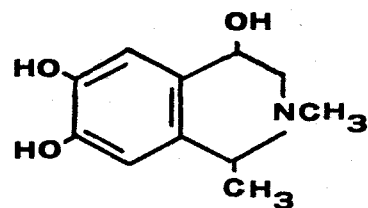
##### II. ANIMALS

Male Sprague-Dawley rats (Holtzman) weighing  $350 \pm 20$  grams were used in all experiments with the exception of the pyrogallol/methanol experiment (vide infra).



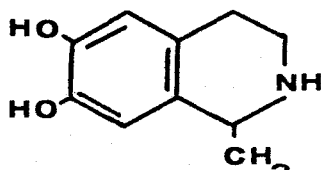
1-methyl 4,6,7-  
trihydroxy-TIQ

( # 1 )



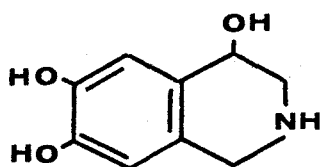
1,2 dimethyl-4,6,7-  
trihydroxy-TIQ (gigantanol)

( # 2 )



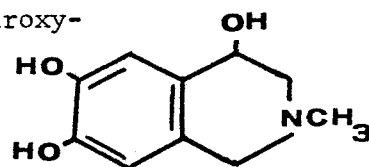
1-methyl-6,7-dihydroxy-  
TIQ (salsolinol)

(# 3)



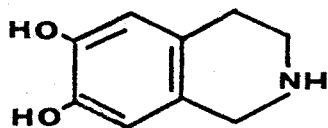
4, 6,7- trihydroxy-TIQ

( # 4 )



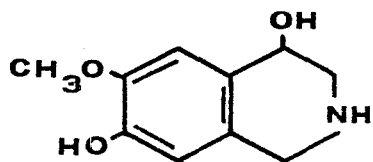
2-methyl-4,6,7-trihydroxy-  
TIQ (1-norgigantanol)

( # 5 )



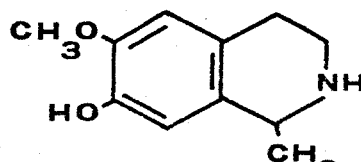
6,7-dihydroxy-TIQ

(# 6)



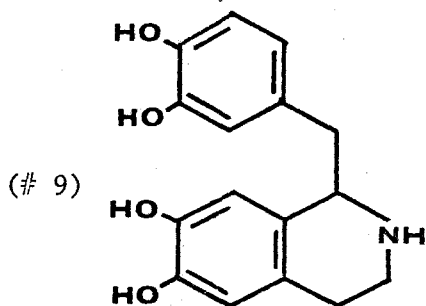
6-methoxy-4,7-dihydroxy-  
TIQ

( # 7 )



1-methyl-6-methoxy  
7-hydroxy-TIQ

( # 8 )



Tetrahydropapaveroline  
(THP)

Fig. 5 1,2,3,4-tetrahydroisoquinolines (TIQs) used in these studies

### III. GAS CHROMATOGRAPHY

The analyses were performed on a Varian Model 2100 GC instrument equipped with electron capture (EC) and flame ionization detectors and injection port extenders. The  $^{63}\text{Ni}$ -EC detector was operated on a direct current mode. Carrier gas was nitrogen ( $\text{N}_2$ ) (zero grade, Liquid Carbonic). U-shaped glass columns (1/8 inch i.d. X 6 feet) were used. All column packings were prepared in our laboratory using a solvent evaporation technique with a rotary flash evaporator (90). The basic solid support was 80/100 mesh Gas Chrom Q (Applied Science Laboratories) coated with various liquid phases: SE-30, Silar, SE-54, OV-17, GE XF-1105 (Applied Science Laboratories), SE-52 (Varian Aerograph), and Dexil 300 (Regis Chemical Company). Carbosieve 60/80 mesh (Supelco, Inc.) or Porapak QS 80/100 mesh (Water Associates, Inc.) were employed for ethanol and AcH determinations. Columns were conditioned at their maximum temperature for 24-36 hours with a  $\text{N}_2$  flow rate of 20 ml/min.

#### B. METHODS

##### I. PREPARATION OF FLUOROACYL DERIVATIVES

To 1 mg of the catechol compound in 5 ml screw capped vials, 0.1 ml acetonitrile and 0.2 ml HFB anhydride or PFP anhydride were added. To each sample of tissue extract a total of 0.2 ml acetonitrile plus 0.4 ml HFB anhydride was added. Each vial was covered with aluminum foil, capped, and left at room temperature. After 30-60 minutes the solution was dried under a  $\text{N}_2$  stream. The vial was then tightly capped and stored at  $-20^\circ\text{C}$  until used for GC

analysis. Dried derivatives obtained in this way were redissolved in distilled ethyl acetate to the appropriate concentration prior to analysis. TIQ #1 and #2 (Fig.5), which were not available as crystalline compounds, were prepared by the method of Cohen and Collins (1) by condensation of AcD with NE and E respectively, and were then derivatized following lyophilization (vide infra). THP fluoroacyl derivatives were prepared from the hydrochloride salt, which was made by dissolving 5 mg THP base in 2 ml 0.05 N HCl in methanol ( 1 : 99 v/v) and evaporating the solution with a N<sub>2</sub> stream. O-methyl metabolites of CAs and TIQs were derivatized in the same manner.

## II. PREPARATION OF CALIBRATION CURVES

In order to determine quantitative measurements and linearity of detector response to catechol compounds, calibration curves were constructed by plotting the peak height versus picogram (pg) substrate. A stock solution (1 mg/ml in ethyl acetate) of each derivative was prepared. In the case of TIQ #1 and TIQ #2, the concentration of each calibration solution was based on the weight of parent amines (E and NE) before reaction. In the situation where the peak areas were measurable, the calibration curves were also prepared by plotting the peak area (planimetric method) versus the concentration, thus permitting comparison of data by two measurements. Calibration curves were determined for each experiment, in order to guard against changes in EC detector response to a particular amine.

### III. EXTRACTION OF CATECHOL COMPOUNDS FROM TISSUE

The entire extraction procedure is summarized in Fig. 6. Each sample of tissue (either whole brain, combined brain parts or pair of adrenal glands) from a single rat was weighed, transferred to a 5-ml conical homogenizing tube (Kontes Glass Company) containing a 2 ml mixture of 1 N perchloric acid and 0.05 M sodium metabisulfite. The sample was homogenized for five minutes while the tube was immersed in an ice-cold bath. All centrifugations were done in the cold room (4°C) in a clinical centrifuge. The homogenate was centrifuged at 2,000 rpm for 15 min. and the resulting supernatant was removed with a Pasteur pipette and was transferred to a 15-ml screw-capped vial. The precipitate was rinsed with another 2 ml of the homogenizing media and was centrifuged as previously described. Both supernatants were pooled and saved, and the precipitate was discarded.  $^{14}\text{C}$ -DA (purified with  $\text{Al}_2\text{O}_3$  prior to use) was added to the supernatant obtained from brain homogenate in order to determine the percent recovery. 10% of the total brain homogenate was examined by scintillation spectroscopy, while the remaining 90 percent of the supernatant was used for  $\text{Al}_2\text{O}_3$  extraction of catechol compounds. Purified  $^{14}\text{C}$ -E was added to the adrenal homogenate for the same purpose and the same steps were followed.

### IV. $\text{Al}_2\text{O}_3$ CATECHOL EXTRACTION PROCEDURE

The pH of the supernatant (above) was brought to between 6-7 by the addition of 1 N NaOH. Activated  $\text{Al}_2\text{O}_3$  (100 mg) was added and pH was adjusted to 8-8.2 by careful addition of 0.2 N NaOH. The mixture was shaken with a mechanical shaker for ten minutes. The  $\text{Al}_2\text{O}_3$  was

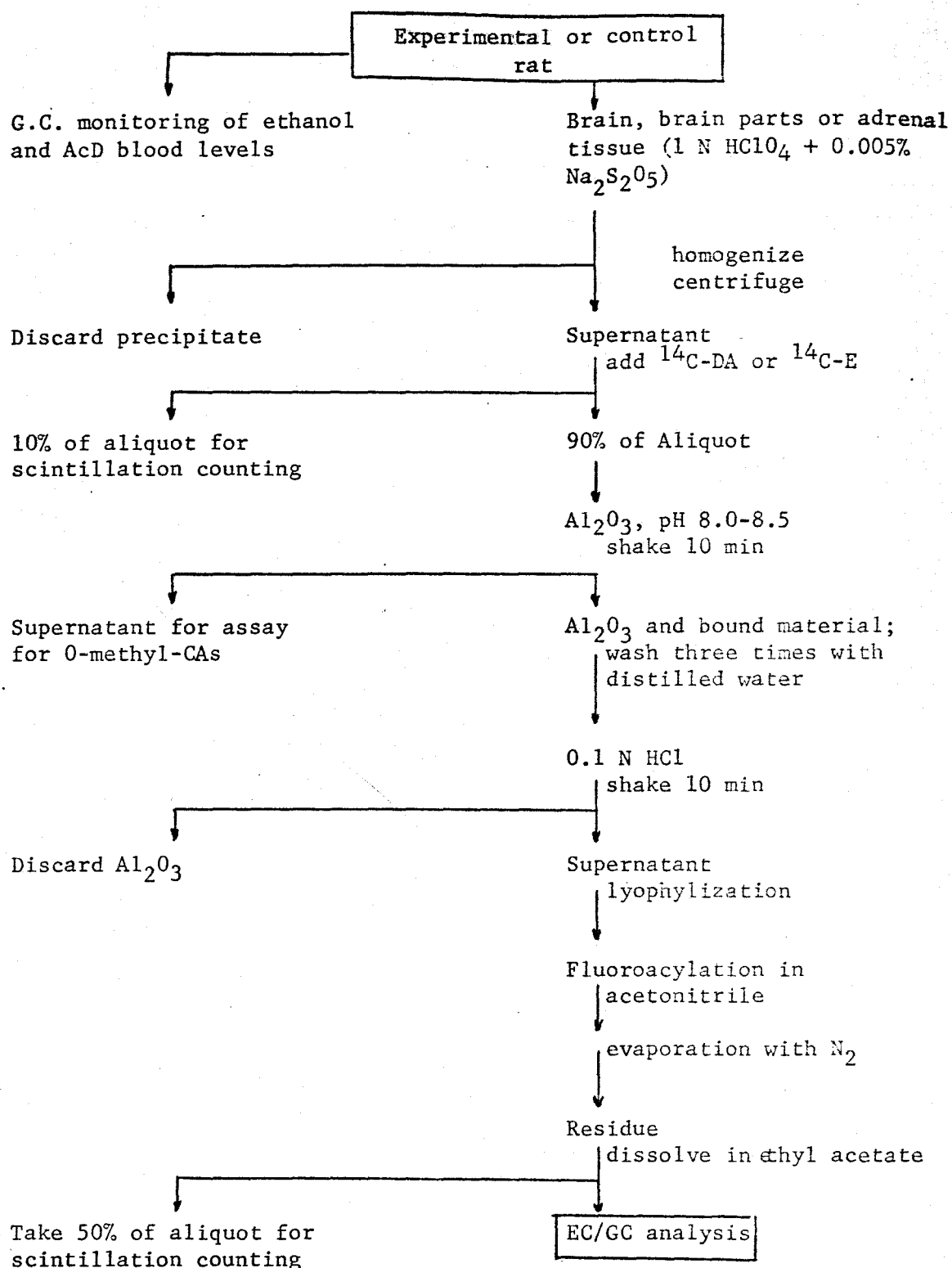


Fig. 6 General Procedure for the Isolation of Tissue Catechols with Activated Aluminum Oxide ( $\text{Al}_2\text{O}_3$ ) and their derivatization for EC/GC analysis.

allowed to settle and supernatant was transferred to another test tube with a Pasteur pipette and stored in the freezer for subsequent analysis of phenolic CA metabolites. The  $\text{Al}_2\text{O}_3$  portion was washed with three 5-ml portions of distilled water. The catechol compounds were then eluted from  $\text{Al}_2\text{O}_3$  by shaking for ten minutes with 2 ml 0.1 N HCl. The  $\text{Al}_2\text{O}_3$  was discarded and the acid supernatant was lyophilized. The dried residue was stored at  $-20^\circ\text{C}$  for later derivatization.

In some instances, a prior purification step was done on brain homogenate. Fatty substances were removed (91) by ten volumes of a mixture of organic solvents (ether-benzene 5:2) added to the supernatant portion of the homogenate. The aqueous layer was then processed through the  $\text{Al}_2\text{O}_3$  purification procedure.

#### V. ION EXCHANGE COLUMN CHROMATOGRAPHY OF PHENOLIC CA METABOLITES

Dowex AG50-WX (50 gm) was washed four times with distilled water and acidified under the flow of eight liters of 2N HCl passing dropwise through a column. The acidified Dowex was brought to neutral pH in the same column with distilled water.

The Dowex, stored under distilled water, was poured into a 4 mm i.d. narrow-tip column until a packing height of ca. 1cm was obtained. The supernatant from the  $\text{Al}_2\text{O}_3$  extraction (the solution containing phenolic CA metabolites) was passed through the column after adjusting the pH to 3.5 with phosphate buffer ( $0.1 \text{ M KH}_2\text{PO}_4 + 0.1 \text{ M NaOH}$ ). The column was washed with four 2 - ml portions of distilled water. The adsorbed materials were then eluted with 2 ml

of a 1:1 mixture of 6N HCl: absolute ethanol (v/v) (121), and the eluant was lyophilized and stored in the freezer for future derivatization.

#### VI. QUANTITATIVE AND QUALITATIVE MEASUREMENT OF PFP AND HFB DERIVATIZED CAs AND TIQs

Since the linearity of the EC detector is limited, the concentration of the HFB or PFP derivatives in ethyl acetate was adjusted to be in the range of pg/ml. However, a problem arose in the quantitation of the suspected TIQ alkaloids in the tissue of the alcohol treated animals, since they were present in such minute concentrations. This necessitated the injection of a concentrated solution of the derivatives, which precluded obtaining precise quantitative results. In previous work (53) it was observed that HFB derivatives were not stable at room temperature in ethyl acetate long enough to complete both quantitative and qualitative measurements on all samples, especially when these investigations require changing GC columns (which then take 12-16 hr to become equilibrated).

Therefore, when the catechol compound from each rat's brain parts or adrenals were extracted and derivatized, the resulting solution was divided into two portions (10% and 90% v/v). Both were dried under a stream of N<sub>2</sub>. The larger portion, to be used for qualitative analysis, was stored at -20°C. The smaller portion was dissolved in 5 ml ethyl acetate. One  $\mu$ l of this diluted solution was analyzed quantitatively for CAs by EC/GC (GE XF-1105) at 4 to 8 x 10<sup>-10</sup> AFS electrometer settings. Peak heights were measured and the concentrations of CAs or their metabolites (un-

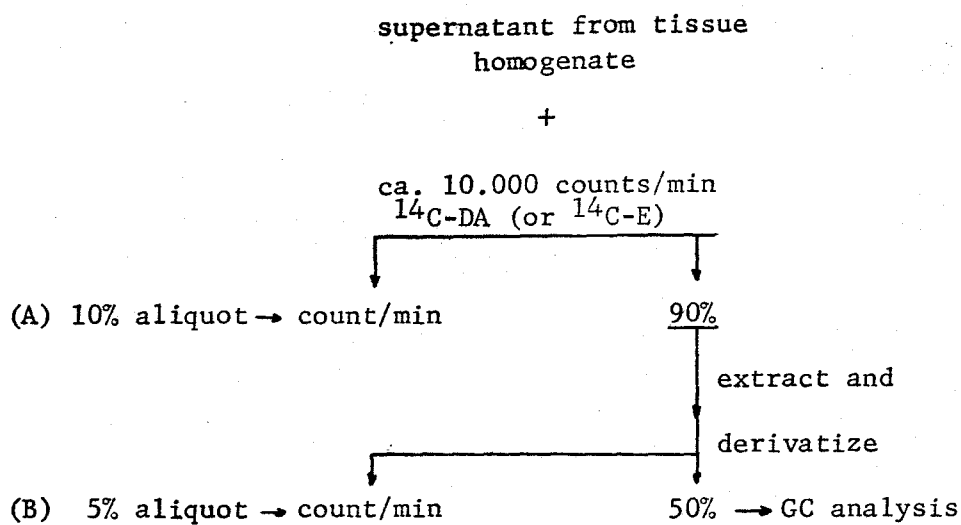


corrected for recovery) per g tissue were calculated using the calibration curves. In the third pyrogallol/acute ethanol experiment (Results, vide infra), adrenal CAs were determined per g protein as well as per g tissue. Adrenal protein was measured spectrophotometrically by the method of Lowry (122).

The larger portion, used for qualitative analysis of potential TIQs at  $1$  to  $4 \times 10^{-10}$  AFS and for radioactive recovery, was dissolved in 1 ml distilled ethylacetate. Approximately 500  $\mu$ l of this solution was utilized to determine overall recovery by scintillation spectroscopy (representative calculation in Fig.7), and 1  $\mu$ l portions of the remaining sample were injected on the GC column (3% OV-17).

#### VII. QUANTITATIVE ANALYSIS OF ACETALDEHYDE AND ETHANOL IN BLOOD

The estimation of AcD and ethanol levels in blood during chronic and acute alcohol intoxication was done by flame ionization GC, modifying the method of Coldwell et al (92). Tail blood was sampled four times during acute intoxication (timing is summarized in Fig. 11) and once at the end of the chronic intubation experiment (two hours before sacrifice). For each sample, 20  $\mu$ l blood was transferred by a heparinized micropipette onto a sodium fluoride impregnated paper disk in a 30-ml serum bottle which was quickly stoppered and kept in ice. Before injecting the sample into the bottles were shaken at 35° C in a thermostatic metabolic shaker for 15 minutes to equilibrate the released gases. Aliquots of 1-2 ml head space gas from the serum bottle were injected on the GC column by means of a gas-tight syringe.



$\% \text{ Recovery} = \frac{2B \times 100}{9 A}$
---

Fig. 7 Calculation of % recovery

#### a. Preparation of Calibration Curves

The calibration curves were prepared by dilution of aliquots of stock solutions of ethanol and of AcD to known concentrations with rat blood. The final concentrations in the standard solutions ranged from 0-3.0 mg/ml for ethanol and 0-80 ug/ml for AcD. The GC parameters were:

Barber Coleman Model 5320 GC:

Column: Aluminum, 6 foot x  $\frac{1}{4}$  inch o.d. packed with Porapak QS (80/100 mesh, Water Assoc.), conditioned for 24 hr at 180° C.

Temps: Column = 145° C, Detector = 200° C

Flow Rates: N<sub>2</sub>, 100 ml/min; H<sub>2</sub>, 30 ml/min; Air, 300 ml/min; and

Varian model 2100:GC:

Column: Glass, 3 foot x 1/8 inch i.d. packed with Carbosieve-B (60/80 mesh, Supelco Co.) conditioned for 24 hr at 250° C.

Temps: Column = 175° C Injector = 230° C Detector = 250° C

Flow Rates: N<sub>2</sub>, 70 ml/min; H<sub>2</sub>, 30 ml/min; Air, 300 ml/min

#### VIII. ACUTE ETHANOL METABOLISM STUDIES

Acute intoxication experiments lasted eight hours each, from 1000 hrs to 1800 hrs. In order to control for circadian rhythm factors, this timing schedule was observed for all short-term experiments. Since it is known that the levels of CAs in rat brain fluctuate diurnally (93), any differences in CAs between experimental and control animals can be assumed to be due to the effect of alcohol.

Drugs and alcohol solutions were prepared in 0.9% (isotonic) saline solution and administered according to the following sched-

ules. After decapitation, combined brain parts, consisting of brain stem, caudate nucleus and midbrain, hypothalamus included, (or whole brain, in the case of the acute ethanol and methanol experiments) and rapidly frozen in liquid N<sub>2</sub> or in dry ice. Organs from each animal were kept in small plastic bags (Whirl-Pak, Nasco) separately at -20°C until homogenization. The above procedures were performed in the cold in less than three minutes.

(a) Ethanol

Each rat received 3 g/kg of 25% (v/v) ethanol in isotonic saline i.p. at 1100, 1350 and 1600 hr, while each control rats each received equivalent volumes of isotonic saline at these times. Two hr after the last injection, the animals were sacrificed and the organs were removed and frozen.

(b) Pyrogallol/Ethanol

In three different studies (December 12, 1972, December 30, 1972, and June 23, 1973), a total of 33 rats were each treated i.p. with 250 mg/kg pyrogallol in isotonic saline at 1000 hr. The 18 experimental animals were given three i.p. injections (3 g/kg each) of 25% ethanol solution (see VIII, a) 60, 210, and 360 min after pyrogallol treatment. Each of the 15 remaining rats received 6 ml of isotonic saline i.p., in place of ethanol.

(c) DOPA/Pyrogallol/Ethanol

In this study, experimental rats were treated as in experiment (b), but with two additional i.p. injections of 100 mg/kg l-DOPA suspended in isotonic saline (4% w/v). The DOPA injections were given 30 and 150 min after pyrogallol; control received equivalent

volumes of isotonic saline at these times.

(d) Pargyline/Pyrogallol/Ethanol

Rats were injected i.p. once on each of two consecutive days with 100 mg/kg pargyline in water (4% w/v). On the second day, one hr after pargyline at 0900 hr, all rats were treated as in experiment (b).

(e) Reserpine/Pyrogallol/Ethanol

Two groups of rats were injected i.p. with either 2.5 mg/kg reserpine (Serpasil, CIBA, 2-cc ampoules of 2.5 mg/cc) or vehicle of Serpasil. On the following day, all were treated with pyrogallol followed by ethanol or saline as in experiment (b)

(f) Pyrogallol/Methanol

The method used with nine animals ( $550 \pm 30$  g) was the same as in Experiment (b), except that the methanol solution in experiment (g) replaced the ethanol solution

(g) Methanol

Rats were given i.p. 3g/kg methanol in isotonic saline (30% v/v) at 1200 hrs, followed by repeat injections at 1430 hr and 1700 hr.

Two hr after the last injection, they were sacrificed. Controls were treated with 3 ml isotonic saline at the same times.

IX. CHRONIC METABOLISM - "ALCOHOLIC" RAT STUDIES

In two chronic intoxication experiments, rats were treated by two different routes (vide infra) with 25% ethanol (v/v) in water for 15 days. Depending on the state of intoxication of the animals, daily amounts ranged from 8-12 g/kg usually in 3 doses/day. They were housed in individual cages and all had free access to

water and, in the case of the chronic i.p. experiment, lab chow. Ethanol was isocalorically substituted by 21% (w/v) dextrose solution in the control animals. During the experiments, food intake and body weight were recorded.

Two different chronic intoxication studies were performed:

a. Gastric Intubation

In this method, ethanol or dextrose solution was administered by gavage with a four-inch urethral catheter attached to a 10-ml syringe. The experiment was started with two intubations/day of ethanol or isocaloric dextrose solution and raised on the 3rd day to 3 intubations (3-4 g/kg) at 0100, 0900 and 1700 hrs, according to Majchrowicz's procedure (77). The average amount of food and water consumed by the ethanol-treated rats was given to the controls the next day (pair-feeding). On the last (15th) day of this study, tail blood samples were collected at ca 1500 hr, to measure the AcD and ethanol levels 2 hr pre-sacrifice.

b. i.p. Injection

The conditions of this experiment were the same as above except for the route of administration and the absence of pair-feeding. In this method, ethanol or dextrose solution was administered via i.p. injection. Along with the ethanol group, another group of rats was injected with the 3g/kg methanol in water (25% v/v). Food and water were offered ad lib. Beginning on the fourth day of the treatments, the animals in both experimental groups began to die. By the xisth day, insufficient numbers of ethanol and methanol injected rats were still alive, and therefore the experiment was discontinued.

### PART THREE

#### RESULTS

##### A. DERIVATIZATION OF CAs AND TIQs

###### I. HFB-DERIVATIVES OF CAs AND SIMPLE TIQs

In previous studies (53), a successful method for the EC/GC analysis of TIQ alkaloids concurrent with their parent compounds, CAs, was developed. Among different methods of derivation, the most suitable results were obtained by 30-60 min reactions of the amines at room temperature with HFB-anhydride in acetonitrile. A single derivative with a distinct retention time was obtained for each CA and TIQ, using 3-5% OV-17 or SE-30 columns. However, E and NE could not be separated on these columns; others have reported this difficulty with TFA derivatives of E and NE on similar column packings (94). Also, some difficulties with the detection and separation of CA metabolites (O-methylated CAs) existed when these liquid phases were used. Therefore, to improve the method, other liquid phases such as SE-52, SE-54, Dexil, Silar and GE XF-1105 were checked at different chromatographic conditions.

With a 5% GE XF-1105 column, excellent separation of nearly all the HFB-derivatives of CAs and their derived TIQs and of phenolic CA metabolites was obtained. Fig.8a illustrates the retention times and relative responses to the EC detector of the HFB-derivatives of (50 pg each) DA, NE, E, salsolinol and 4,6,7-trihydroxy-TIQ, as well as the 1-methyl-TIQ reaction products of E and NE with Ach. Of special note is the separation of E, NE and DA (HFB derivatives),

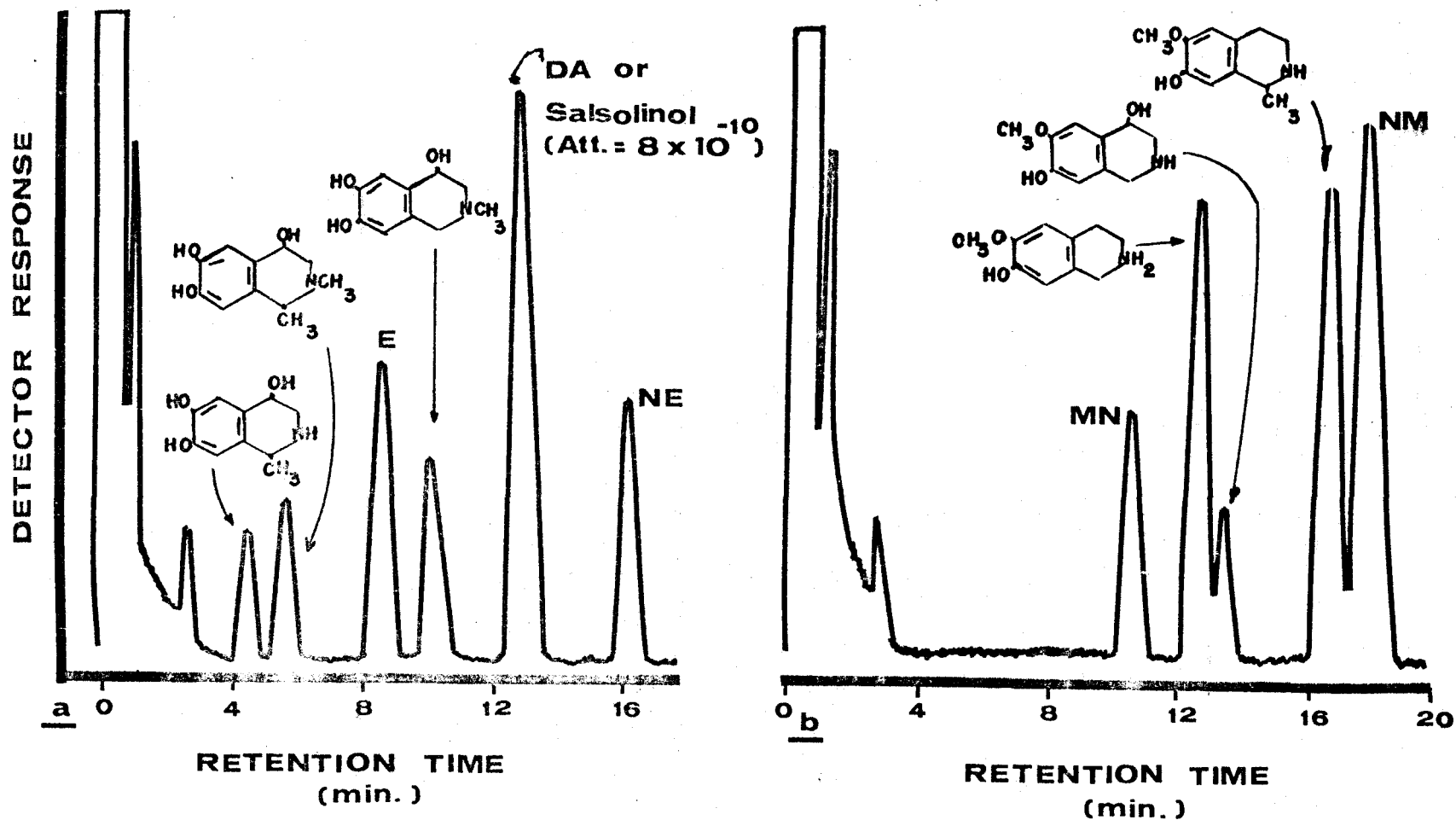


Fig. 8. EC/GC Chromatograms of the HFB derivatives of 50 pg of the CAs and three TIQ products (a) and of the O-methylated derivatives of the CAs and two TIQ products (b).

GC conditions: 5% GE XF=1105 on gas chrom Q; Column Temp.=175°C; N<sub>2</sub>, 35 ml/min; Att.  $4 \times 10^{-10}$  AFS



which is of significance in adrenal gland analyses. HFB-DA and HFB-salsolinol were not separated on GE XF-1105. Therefore a 3% OV-17 column, which readily separates these two derivatives, was used in future analyses of brain extracts.

The minimum detectable quantity (MDQ), i.e., the amount of sample which gives a detector response equal to twice the noise level, consistently was smaller on 5% GE XF-1105 for most HFB-amines than on any other liquid phase. Unusually sensitive were HFB-DA (MDQ= 0.5-1 pg) and HFB-salsolinol (MDQ=0.2-0.5 pg).

Fig. 8b is a chromatogram obtained by separation of 50 pg each of several O-methylated metabolites of CAs and TIQs on a 5% GE XF-1105 column.

## II. PFP-DERIVATIVES OF CAs AND SIMPLE TIQs

Table 2 illustrates the retention times of the PFP derivatives of some catechol compounds on different columns such as SE-52, SE-54 and GE XF-1105. The relative EC detector responses to the PFP-derivatives are shown in Table 2. SE-30 and SE-54 were acceptable phases for resolution of a mixture of CAs and several TIQ derivatives with appreciable sensitivity. Overall, although direct comparisons were not done, the HFB- derivatives appeared to show more than 40-60% greater sensitivity to EC detection than the PFP derivatives, in agreement with Arnold and Ford (83).

## III. DERIVATIZATION OF THP

Attempts to derivatize crystalline THP (free base) with HFB or PFP anhydride in ethyl acetate or acetonitrile were unsuccessful under the usual conditions. However, the hydrochloride salt of THP

Table 2

Retention times and EC/GC responses of PFP-derivatives of CAs and TIQs on various stationary phases

Stationary <sup>1</sup> Phase	GC Conditions <sup>2</sup>	Retention Time (min)				Sensitivity <sup>3</sup>			
		DA	NE	E	Salsolinol	DA	NE	E	Salsolinol
3% SE-30	A	3.30	4.50	5.00	--	0.12	0.28	0.25	--
5% SE-52	A	4.10	2.45	--	6.00	0.20	0.25	--	0.18
5% SE-54	A	4.10	2.50	2.50	6.45	0.20	0.30	0.25	0.10
3% OV-17	B	4.20	2.30	2.30	6.45	0.15	0.20	0.18	0.15
5% GE XF-1105	C	7.40	9.10	5.15	7.40	0.10	0.25	0.20	0.10

1. On 80/100 Gas Chrom Q.

2. A: N<sub>2</sub> Flow Rate= 30 ml/min; T = 160°CB: N<sub>2</sub> Flow Rate= 30 ml/min; T = 170°CC: N<sub>2</sub> Flow Rate= 35 ml/min; T = 175°C3. ng amines which give 50% deflection at  $4 \times 10^{-10}$  AFS.

was readily derivatized with these reagents; the derivatives were examined on different columns (Table 3). Analogous to the other catechol compounds, the HFB-derivative of THP demonstrated a greater EC sensitivity than the PFP derivative. Relative sensitivity and retention times of the two fluoroacyl THP derivatives are shown in Table 3. It was observed that PFP-THP sensitivity was strikingly low on all columns, with an MDQ of 200-250 pg (or sensitivity, as defined in table 3, of 2-5 ng).

#### B. CALIBRATION CURVES FOR CAs AND TIQs

Calibration curves were constructed for DA, NE, E, salsolinol and 4,6,7 trihydroxy TIQ on 5% GE XF-1105 following HFB anhydride/ acetonitrile derivatization. The curves in Fig. 9 are the averages (S.D. = 8.5%) of twelve sets of these calibration experiments. Injection of a constant volume (1 ul of series of dilutions of standards) resulted in peak heights proportional to the concentrations. Such linearity could hardly be demonstrated with injection of increasing volumes of a single standard. Upper limits of linearity ranged between 200-500 pg depending on the substrate. This upper limit was lowest for those compounds which demonstrated the highest EC responses. The calibration curves (not shown) for HFB-derivatives of O-methylated catechols, 3-methoxy-tyramine, metanephine, normetanephine, 6-methoxy-4-7-dihydroxy-TIQ (#7 in Fig. 5 ) and 1-methyl-6-methoxy-7-hydroxy-TIQ (#8 in Fig. 5 ) were also prepared using the same conditions as the catechol calibration curves and were found to be linear up to 400-800 pg, depending on the substrate amine.

Table 3

A comparison of retention times and relative EC/GC sensitivities of fluoroacyl derivatives of THP on different liquid phases<sup>1</sup>

Stationary Phase	PFP-THP		HFB-THP	
	Retention Time(min)	Sensitivity <sup>2</sup>	Retention Time(min)	Sensitivity <sup>2</sup>
3% OV-17	7.40	5.2	8.55	4.0
5% SE-54	11.10	2.5	16.10	2.0
3% SE-30	13.30	2.5		

<sup>1</sup>GC Condition: Flow rate, 30 ml/min; column temperature, 190°C,

<sup>2</sup>ng amines which give 50% deflection at  $4 \times 10^{-10}$  AFS

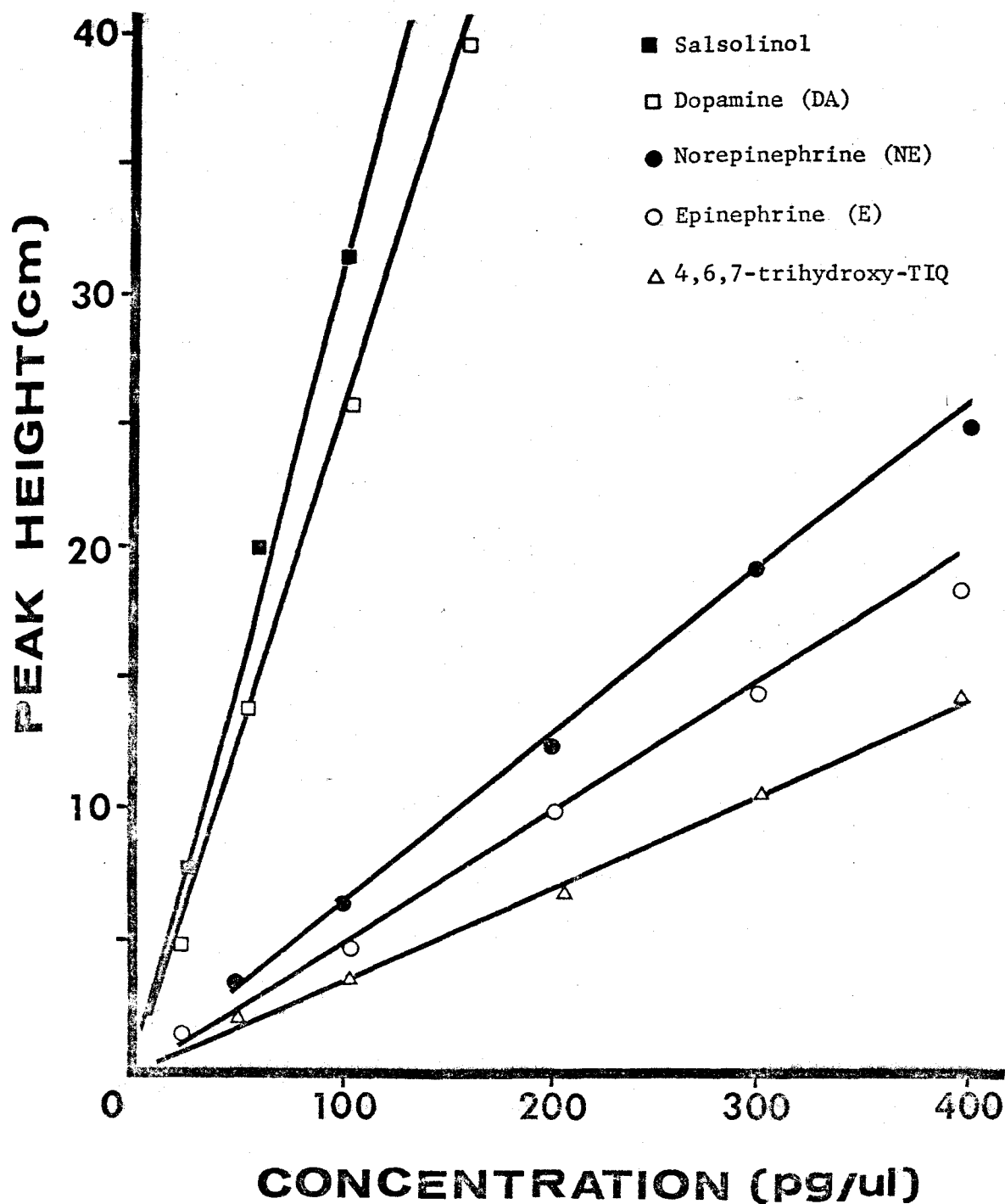


Fig. 9. EC/GC calibration curves for NE, E, the HCl salts of DA and 4,6,7, trihydroxy-TIQ, and the HBr salt of salsolinol

GC conditions: 5% GE XF-1105; column temp. 175°C, N<sub>2</sub> = 35 ml/min  
Att. = 4 x 10<sup>-10</sup> AFS

### C. EXTRACTION AND RECOVERY

The extraction procedure described in experimental Fig. 6 involves the adsorption of catechol compounds on  $\text{Al}_2\text{O}_3$  and their elution with hydrochloric acid. This is a modification of the method studied in detail by Anton and Sayre (89).

Previously it was found (53) that 100 mg activated aluminum oxide was optimum for EC/GC determination (HFB-derivatives) of 1 mg CA or TIQ in aqueous solution. In these experiments this amount was determined to be adequate for the extraction of a pair of rat adrenal glands or of one brain sample.

The highest recovery was obtained when elution of tissue CA mixtures with added  $^{14}\text{C}$ -DA or  $^{14}\text{C}$ -NE was carried out with 0.1 N HCl and when derivatization followed the lyophilization of eluant (table 4). "Direct derivatization" involving HFB anhydride/acetonitrile treatment of catechols in the alumina-bound state was an alternative acid-free procedure developed in M.S. degree research (53). Those original studies with non-radioactive substrates indicated that recoveries were adequate. However, recent experiments with  $^{14}\text{C}$ -substrate showed that overall recoveries by direct derivatization were about 10% (Table 4). This direct derivatization method was not improved or utilized further for tissue studies.

When aliquots of brain homogenate containing increasing amounts of added NE, DA, salsolinol and 4,6,7-trihydroxy-TIQ (#4 in Fig.5) were carried through the entire procedure of adsorption on aluminum oxide and elution with 0.1 N HCl, followed by derivatization, the calibration curves very similar to Fig.9 were obtained. Recoveries

Table 4

% Recovery (mean  $\pm$  S.D.) of  $^{14}\text{C}$ -CAs<sup>1</sup> following activated  $\text{Al}_2\text{O}_3$  extraction and derivatization with HFB-anhydride/acetonitrile

	Direct derivatization of lyophilized, $\text{Al}_2\text{O}_3$ -bound CAs (No acid elution)	Elution of CAs from $\text{Al}_2\text{O}_3$ with 0.1 N HCl, lyophilization, and derivatization	
		CAs in 1N $\text{HClO}_4$	CAs added to rat brain homogenate
$^{14}\text{C}$ -DA	9.05 $\pm$ 0.80 (3)*	58.9 $\pm$ 2.8 (3)	42.9 $\pm$ 4.1(9)
$^{14}\text{C}$ -NE	15.25 $\pm$ 0.65 (3)	87.1 $\pm$ 1.0 (3)	—
$^{14}\text{C}$ -E	11.46 $\pm$ 0.56 (3)	—	75.2 $\pm$ 10.2(10)

<sup>1</sup>  $^{14}\text{C}$ -CAs purified on  $\text{Al}_2\text{O}_3$  prior to their addition to aqueous solution or homogenate

\* Number of determinations

(for  $^{14}\text{C}$ -CAs) from tissue agree with those from 1 N  $\text{HClO}_4$  solution (Table 4).

#### D. EC/GC ESTIMATION OF CAs IN TISSUE

##### I. BRAIN

The whole brains of four rats each analyzed on two different columns (5% GE XF-1105 and 5% SE-54). In Table 5 are shown our results of these analyses, compared with the values determined by other investigators using EC/GC and spectrofluorometric methods.

##### II. ADRENAL GLANDS

The CA concentrations in the adrenal glands of four rats, determined on 5% GE XF-1105, are shown in Table 6. Our findings (Table 6) are compared with the results reported by Imai et al (95), who also used an EC/GC method (TFA derivatizations). Literature values for adrenal E determined spectrofluorometrically, somewhat higher than EC/GC values, are also included in Table 6.

#### E. BLOOD ETHANOL AND ACETALDEHYDE LEVELS IN VIVO

##### I. CALIBRATION CURVES

A Porapak QS column in a Barber Coleman 5320 flame ionization GC was employed for the estimation of AcD and ethanol concentrations. Using the microtechnique described in Experimental, two different calibration curves were prepared on Porapak QS, one for AcD concentrations ranging between 0.5-5 ug/ml and a second curve for AcD concentrations ranging from 10-80 ug/ml. A carbosieve-B column in the Varian 2100 GC was unresponsive to low AcD levels but showed sensitivity to the relatively high AcD levels (10 ug/ml blood). Therefore, Carbosieve-B was used only to verify blood AcD identity



Table 5

Comparison of EC/GC and spectrofluorimetry (SF) estimations of CA concentrations in whole rat brain

Method	N	ug/g tissue $\pm$ S.D.		Reference
		NE	DA	
EC/GC on 5% SE-54 *	4	0.55 $\pm$ 0.08	0.90 $\pm$ 0.07	this dissertation
EC/GC on 5% GE XF-1105**	4	0.46 $\pm$ 0.08	0.99 $\pm$ 0.09	this dissertation
EC/GC on 2% GE XF-1105	5	0.57	0.81	(95)
SF	10	0.45 $\pm$ 0.01	1.00 $\pm$ 0.08	(96)
SF		0.45 $\pm$ 0.01	0.96 $\pm$ 0.01	(97)

\* GC conditions: column temperature, 160°C;N<sub>2</sub> flow rate, 30 ml/min\*\* GC condition : column temperature, 175°C;N<sub>2</sub> flow rate, 35 ml/min

Table 6

Comparison of EC/GC and spectrofluorimetry (SF) estimations of CA concentrations in rat adrenal glands

Method	N	ug/g tissue $\pm$ S.D.			Reference
		NE	DA	E	
EC/GC	4	173 $\pm$ 35	11.0 $\pm$ 2	504 $\pm$ 66	this dissertation
EC/GC	7	190	4.6	520	(95)
SF	10	160 $\pm$ 6		899 $\pm$ 65	(102)
SF	10	254 $\pm$ 16		951 $\pm$ 50	(96)

\*: GC conditions: 5% GE XF-1105; column temperature, 175; N<sub>2</sub> flow rate, 35 ml/min

in pyrogallol/ethanol rats. Fig. 10 shows Porapak calibration curves (S.D.  $\pm$  11.5%) prepared for the estimation of blood AcD.

## II. BLOOD ETHANOL AND ACETALDEHYDE LEVELS DURING PYROGALLOL/ACUTE ETHANOL METABOLISM

In two separate experiments, blood AcD levels in pyrogallol pre-treated, ethanol injected rats were estimated and compared to the levels in rats treated with ethanol only. Fig. 11 shows the concentrations/time curves of AcD in these two ethanol groups. 20 min after i.p. ethanol, the AcD blood level in "ethanol only" rats was  $2.8 \pm 0.9$  ug/ml. In the pyrogallol/ethanol group the concentration of AcD at this time was potentiated to  $41 \pm 12$  ug/ml blood and remained elevated after five hours. Thus, pyrogallol pretreatment elevates ethanol derived AcD ca. 10-15 fold, in agreement with other work in this laboratory (105).

Blood ethanol in both groups was determined 20 min after i.p. ethanol and found to be elevated slightly but non-significantly in the pyrogallol/ethanol group;  $2.40 \pm 0.68$  mg/ml compared to  $1.95 \pm 0.52$  mg/ml in "ethanol only" rats.

## III. BLOOD ETHANOL AND ACETALDEHYDE LEVELS DURING CHRONIC ETHANOL METABOLISM (INTUBATION)

An estimate of blood ethanol and AcD in rats chronically intubated with ethanol (the alcoholic rat experiment) was obtained on the 15th and last day of intoxication, 2 hr before sacrifice (5-6 hr after the last ethanol intubation at 0900 hr). The average values of  $3.9 \pm 0.9$  ug/ml and  $1.6 \pm 0.75$  mg/ml for blood AcD and ethanol, respectively, were obtained for six rats, agreeing with the results

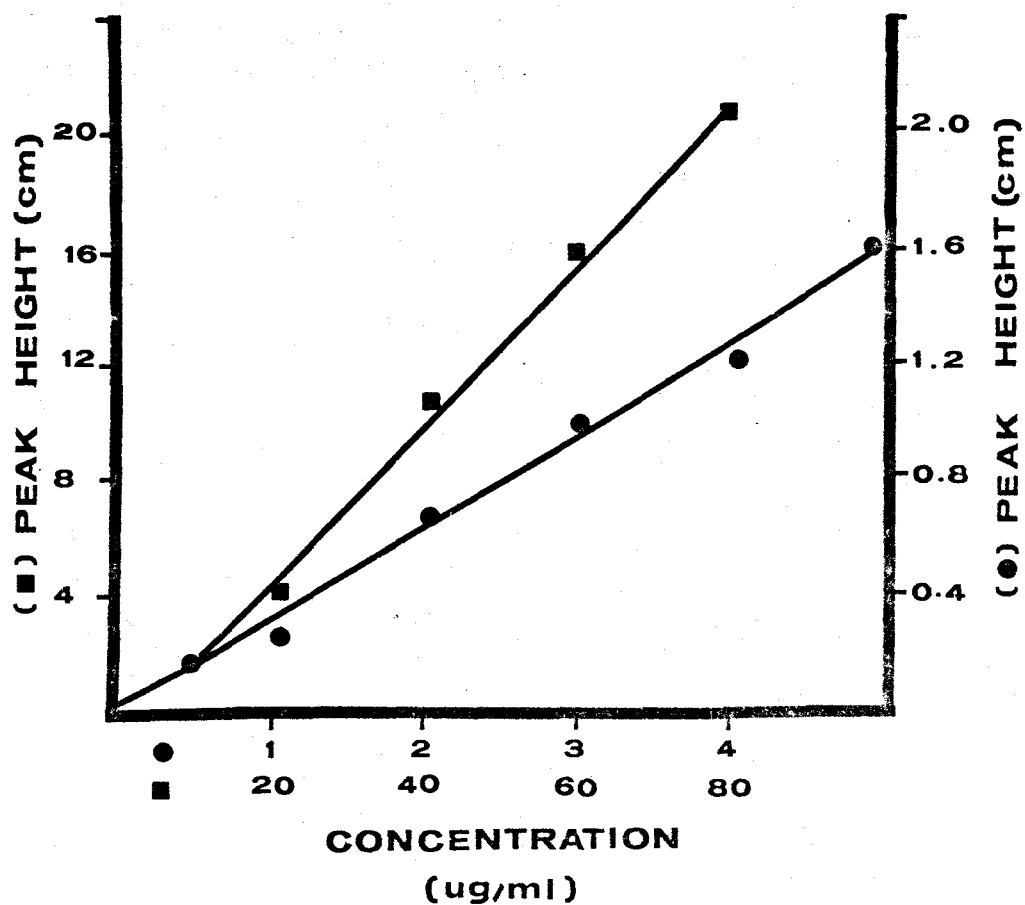


Fig. 10. Calibration curve for AcD in whole blood determined by the micromethod of Coldwell et.al. ( 92 )

GC conditions: Porapak QS; column T. 130°C, N<sub>2</sub>, 100 ml/min

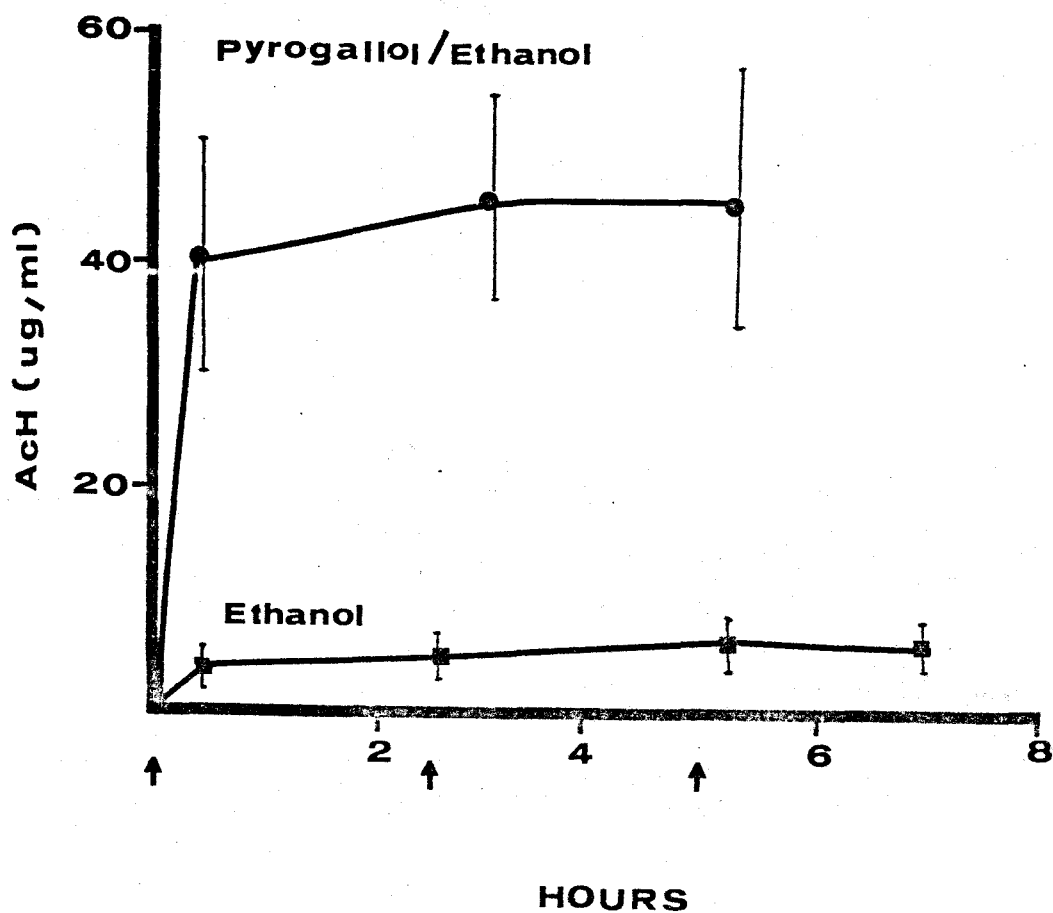


Fig. 11 Blood AcD concentrations following i.p. ethanol (3 g/kg, at times indicated by arrows) in pyrogallol pretreated (●) or untreated (■) rats.

Conditions: Porapak Qs,  $t=130^{\circ}\text{C}$ ,  $\text{N}_2$ , 100 ml/min

of Majchrowicz (77). The results are compared with literature values in Table 7.

## F. ACUTE ETHANOL METABOLISM

### I. FOLLOWING PYROGALLOL PRETREATMENT

#### a. EC/GC Analyses of Combined Brain Parts (brain stem, caudate nucleus and midbrain, including hypothalamus)

Fig. 12, Chromatogram A, is a normal GC chromatogram ( on 3% OV-17) of the combined brain parts from a control (pyrogallol/saline) rat. The consistent appearance of a component (hatched peak) with a retention time identical to salsolinol, the TIQ derived from DA and AcD, was observed in the brain parts of each pyrogallol/ethanol-treated rat (Chromatogram B), and was not observed in control samples. This new component was clearly augmented when HFB-salsolinol was added to the GC injection solution (Chromatogram C, Fig. 12).

The proof that this new compound did not result from reaction of AcD during workup is supported by its absence from control brain samples to which was added AcD (100 ug/ml) during homogenization. Further more, the retention times of several catechol compounds which possibly could be present in the experimental brain and could contribute to this peak agreeing with salsolinol were determined. Table 8 shows that the HFB-derivatives of these compounds have retention times quite different from salsolinol and the new component in the experimental brain parts.

To further verify the identity of salsolinol in the brain parts of pyrogallol/ethanol animals, the experiment was repeated a fourth time with nine rats. Combined brain part extracts were derivatized

Table 7

Blood ethanol and AcD concentrations<sup>1</sup> in the rat and human during chronic ethanol intoxication

Subject	Route of ingestion	Amount/day (g/ kg)	Duration of intoxication (days)	Range		Reference
				Ethanol (mg/ml)	AcD(ug/ml)	
rat	gastric intubation	8-12	15	120-350	2.7-5.1	this dissertation
rat	gastric intubation	12-15	8	50-300	1.0-3.0	(77)
human	free drinking	32 ounce of 50% grain ethanol	7-10	100-400	1.0-2.5	(98)

1. Determined by different GC methods

# LEGEND FOR FIGURE 12

EC/GC chromatogram of the HFB-catechol-containing compounds in the combined brain parts of pyrogallol-treated rats following acute ethanol intoxication (chromatogram B) or saline (chromatogram A).

Chromatogram (c) shows the addition of 50 pg authentic HFB-salsolinol to the chromatogram (B) injection solution.

GC conditions: 3% OV-17 on 80/100 Gas Chrom Q; column temp.=160°C; N<sub>2</sub>, 30 ml/min. Att.  $2 \times 10^{-10}$  AFS, except for DA in the control, which is  $4 \times 10^{-10}$  AFS



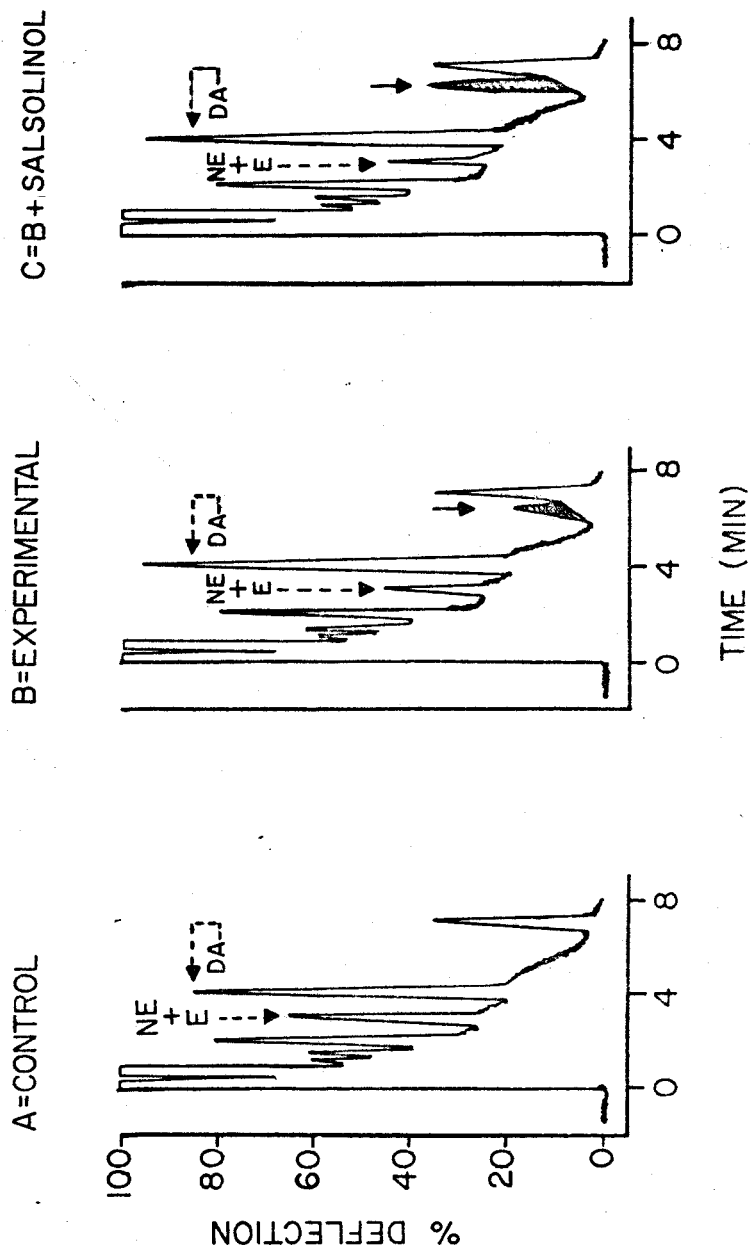


Fig. 12

Table 8

Comparison of EC/GC retention times<sup>1</sup> of known catechol compounds to the unknown component detected in CA-rich brain parts of pyrogallol/ethanol rats

Compound (HFB-derivative)	Retention time (min)
Unknown substance in experimental brains	6.45
Salsolinol	6.45
Epinine	3.50
N-acetyl-DA	2.75
3, 4-dihydroxy phenyl glycol	1.50
3, 4-dihydroxy phenyl ethanol	1.30
3-methoxy-catechol	1.30
Pyrogallol	1.00

<sup>1</sup> 3% OV-17; column T, 160°C; Flow rate, 30 ml/min

with PFP-anhydride in acetonitrile and were analyzed on SE-54. A component (shaded peak, Fig. 13) with a retention time identical to PFP-salsolinol was observed in each experimental (pyrogallol/ethanol) brain sample (h=5) but was not observed any of four control brain parts. Addition of authentic PFP-salsolinol to experimental samples increased the height of this new peak.

In Table 9 the effect of acute ethanol (7 hr metabolism) on the CA concentrations and salsolinol formation in the combined brain parts of rats treated with pyrogallol is shown. The concentration of salsolinol in the brain parts of all experimental rats averaged from samples (three experiments) was  $17 \pm 5.3$  ng/g combined brain parts. As the data shows, DA and NE concentrations in the combined brain parts of pyrogallol rats were depressed 45-55% and 30-40%, respectively, by the ethanol treatment.

Interestingly, the presence of a substance identical to E in the brains of all pyrogallol-treated animals was noted (Table 9) when brain extracts were analyzed on 5% GE XF-1105 for CA quantitation. Assuming this material is E, a higher concentration ( $83 \pm 20$  ng/g brain parts) was seen in the experimental rats than in the control ( $66 \pm$  ng/g brain parts), but the increase was not statistically significant.

Another interesting finding was the fact that an unidentified component with an unusually long retention time (LRT) on GE XF-1105 of 60-70 min ("LRT" component), observed in all brain samples from pyrogallol/saline controls, was nearly absent from all pyrogallol/

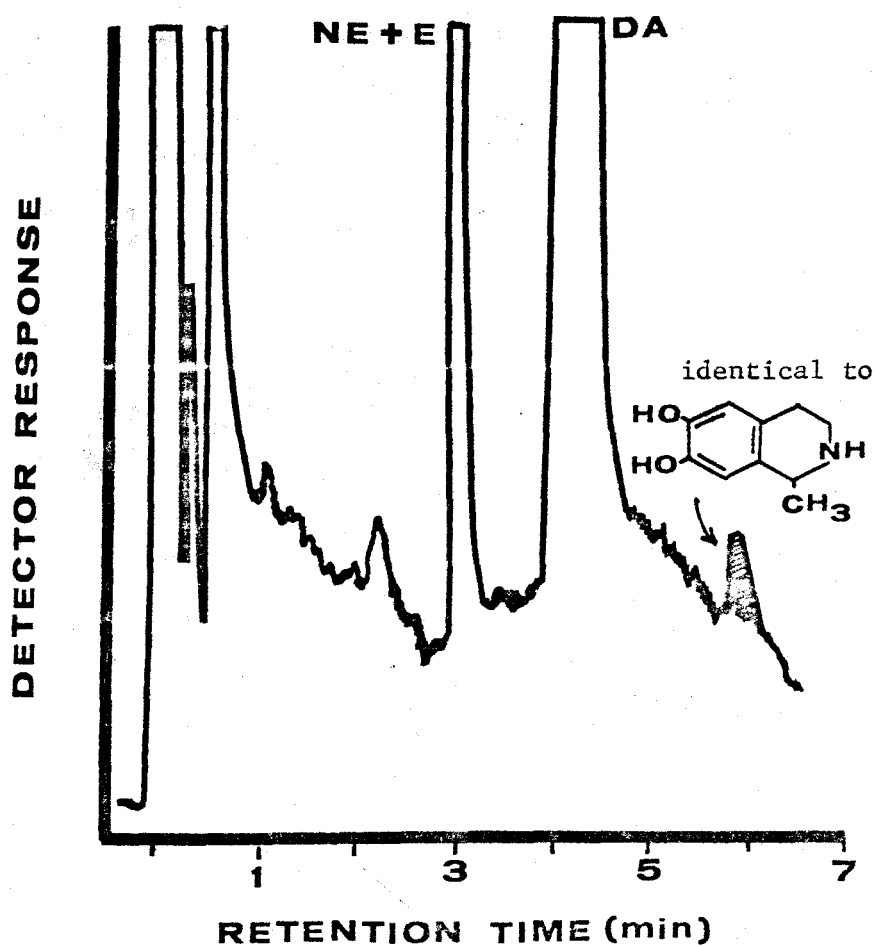


Fig. 13. EC/GC chromatogram of the PFP-catechol-containing compounds in the combined brain parts of pyrogallol-treated, acute ethanol-injected rats. Hatched peak identical to authentic PFP-salsolinol is absent from brain parts of saline control rats.

GC condition : 5% SE-54 on 80/100 Gas Chrom Q; Column Temp.= 160°C,  
N<sub>2</sub> - 30 ml/min, Att.= 2 x 10<sup>-10</sup>AFS

Table 9

Effect of acute ethanol on salsolinol formation and CA concentrations<sup>1</sup> in combined brain parts<sup>2</sup> of pyrogallol-pretreated rats

Experiment No.	Drug Treatment <sup>3</sup>	ug/g tissue <sup>+</sup> S.D.				Salsolinol
		DA	NE	E		
1	Pyrogallol/saline (4)	1.92 $\pm$ 0.21	0.80 $\pm$ 0.09	0.06 $\pm$ 0.01		not detectable
	Pyrogallol/ethanol (5)	0.87 $\pm$ 0.39*	0.63 $\pm$ 0.20	0.07 $\pm$ 0.01		0.018 $\pm$ 0.009
2	Pyrogallol/saline (6)	1.98 $\pm$ 0.17	0.98 $\pm$ 0.16	--	--	not detectable
	Pyrogallol/ethanol (6)	1.05 $\pm$ 0.15*	0.63 $\pm$ 0.07	--	--	0.017 $\pm$ 0.004
3	Pyrogallol/saline (5)	2.06 $\pm$ 0.26	1.02 $\pm$ 0.15	0.06 $\pm$ 0.01		not detectable
	Pyrogallol/ethanol (7)	1.06 $\pm$ 0.10*	0.60 $\pm$ 0.07*	0.08 $\pm$ 0.02		0.015 $\pm$ 0.003

1. Values are corrected for recoveries based on <sup>14</sup>C-DA recovery (range: Exp. 1, 42.3% - 50.1%; Exp. 2, 39.8% - 47.5%; Exp. 3, 40.9% - 53.0%)

2. Combined brain stem, caudate nucleus and midbrain including hypothalamus

3. Number of rats/group in parenthesis

\*  $p < 0.01$  compared to the pyrogallol/saline control

ethanol brain samples. Although it could not be quantitated, this LRT component in the controls was a significant component in the HFB-derivatized extracts, as judged by the area of its peak (70% deflection) at  $8 \times 10^{-10}$  AFS.

b. EC/GC Analysis of Adrenal Glands

EC/GC qualitative results of adrenal glands of rats treated with pyrogallol/ethanol, obtained on 5% GE-XF-1105, are presented in Fig.14. A new (shaded) peak on the chromatograms, with a retention time identical to the major E-derived TIQ, gigantanol, was observed in all adrenal glands from 18 experimental rats (pyrogallol/ethanol) but not in adrenals from control (pyrogallol/saline) rats. This peak was potentiated when the HFB-derivative of gigantanol from E/ACh reaction mixtures was added to the injection solutions. The average estimated concentration of biosynthesized gigantanol in 13 pairs of adrenal glands (three experiments) was  $3.7 \pm 0.49$  ug/g tissue, corresponding to ca. 1-2% of the E levels in the experimental animals.

E levels in adrenal glands underwent significant changes due to pyrogallol/ethanol, as they decreased by 50-60% (Table 10). A non-significant 10% decrease, but nevertheless a definite trend, was also observed in NE. Conversely, DA levels were slightly increased, but this change was not significant.

II. FOLLOWING PARGYLINE-PYROGALLOL PRETREATMENT

a. EC/GC Analysis of Combined Brain Parts

The combined brain parts of rats treated with two doses of pargyline, 24 hr and 1 hr prior to the acute pyrogallol/ethanol or pyrogallol/saline administration, were analyzed for HFB-salsolinol

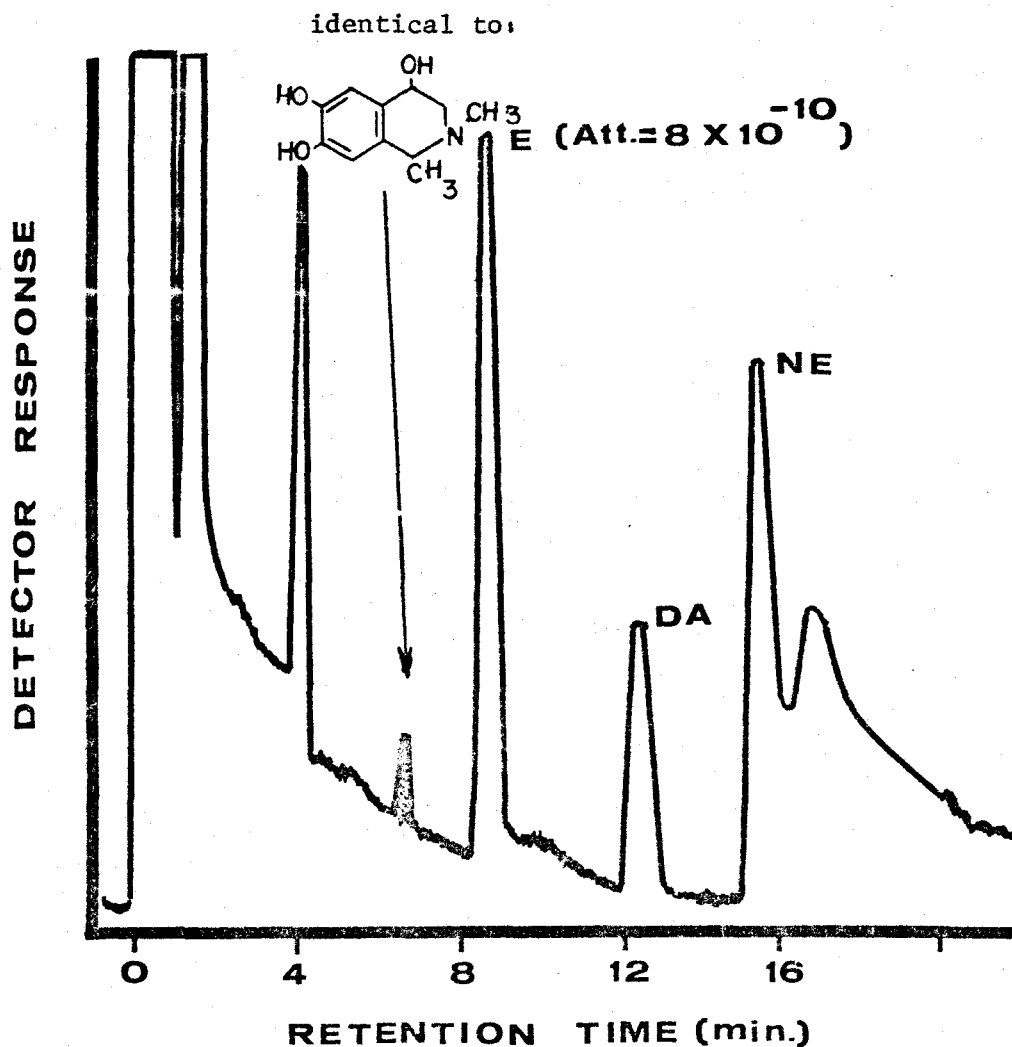


Fig. 14. GC chromatogram of the HFB-catechol containing compounds in the adrenals of pyrogallol-treated, acute ethanol-intoxicated rats. Hatched peak identical to authentic HFB-gigantanol is absent from adrenals of saline control rats.

GC conditions: 5% GE XF-1105 on 80/100 Gas Chrom Q; column temp. =  $175^{\circ}\text{C}$ ;  $\text{N}_2$ , 35 ml/min; Att. =  $2 \times 10^{-10}$  AFS

Table 10

Effect of acute ethanol on gigantanol formation and CA concentrations<sup>1</sup> in adrenal glands of pyrogallol-pretreated rats

Experiment <sup>2</sup> No.	Drug Treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.			Gigantanol <sup>4</sup>
		DA	NE	E	
1	Pyrogallol/saline(4)	14.4 $\pm$ 4.0	190 $\pm$ 58	560 $\pm$ 18	not detectable
	Pyrogallol/ethanol(5)	14.0 $\pm$ 4.9	119 $\pm$ 48	301 $\pm$ 62*	3.7 $\pm$ 0.66
2	Control (5)	10.3 $\pm$ 1.7	146 $\pm$ 22	425 $\pm$ 46	not detectable
	Experiment (7)	15.2 $\pm$ 2.1*	125 $\pm$ 18	171 $\pm$ 37*	4.0 $\pm$ 1.05
3	Control (6)	9.7 $\pm$ 2.0	168 $\pm$ 35	434 $\pm$ 41	not detectable
	Experiment (6)	12.5 $\pm$ 3.2	138 $\pm$ 19	224 $\pm$ 39*	3.1 $\pm$ 0.77

1. The values are corrected for recoveries based on <sup>14</sup>C-E recovery (range, Exp.1, 59.8% - 75.2%; Exp. 2, 61.0% - 69.7%; Exp. 3, 64.7% - 74.4%)
  2. The CA concentrations in exp. 3 were calculated for per g protein in addition to per g tissue as shown and the results in both cases were similar (see appendix II)
  3. Number of rats/group in parenthesis
  4. This figure at best should be considered a minimum estimate, because the aqueous E-AcD condensation itself forms 30-40% of supposedly other gigantanol isomers (1) (which were not separable in GC of adrenals), and because the calibration curve used for estimation was actually based on quantity of starting amine (E) rather than on major product (gigantanol)
- \*  $p < 0.01$  compared to the pyrogallol/saline control



on 3% OV-17. The chromatograms were very similar to those from the pyrogallol/ethanol experiments. A component corresponding to salsolinol was observed (Table 11) in all pargyline-pretreated, pyrogallol/ethanol rats, in significantly higher concentrations than in the pyrogallol/ethanol brain samples (Fig.15). The component was absent in the brain parts of three out of the seven rats treated with pargyline/pyrogallol/saline (controls) but, interestingly, the remaining four controls demonstrated an extremely small quantity (2-5 ng/g brain parts) of a component with a retention time identical to that of salsolinol.

Again the large peak area due to the LRT component was present in the control brain samples and was significantly diminished in or absent from the experimental brain parts. No effort was made to quantify the LRT component and compare its area to other experiments.

As shown previously in Results, EC/GC is much less responsive to the DA-derived benzyl-TIQ than to salsolinol. But it is reasonable to expect the formation of the benzyl-TIQ in this experiment because MAO inhibition has been known to increase both DA and THP biosynthesis in vitro (10). However, when pargyline/pyrogallol/ethanol brain parts were examined on 3% OV-17 at 190°C, conditions at which HFB-THP chromatographs at 8.55 min, there was no evidence in any experimental samples for THP.

Brain CA concentrations were estimated on 5% GE XF-1105. Table 11 shows the expected increase in DA and NE levels in the combined brain parts of the control pargyline/pyrogallol/saline rats (by 130% and 57% respectively) compared to the pyrogallol/saline control rats

Table 11

The effect of acute ethanol on salsolinol formation and the CA concentrations<sup>1</sup> in the combined brain parts<sup>2</sup> of pargyline/pyrogallol-treated rats

Drug Treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.		Salsolinol
	DA	NE + E <sup>4</sup>	
Pargyline/Pyrogallol/ Saline (4)	4.44 $\pm$ 0.42	1.26 $\pm$ 0.20	not detectable
Pargyline/Pyrogallol/ Ethanol (7)	3.27 $\pm$ 0.28**	0.68 $\pm$ 0.14*	0.118 $\pm$ 0.020

1. The values are corrected for recoveries based on <sup>14</sup>C-DA recovery (range, 40.8% - 49.6%).

2. Combined brain stem, caudate nucleus and midbrain, including hypothalamus.

3. Number of determinations

4. The values include both NE and E because 3% OV-17 column does not separate these two HFB-amines.

\* p < 0.01

\*\* p < 0.001

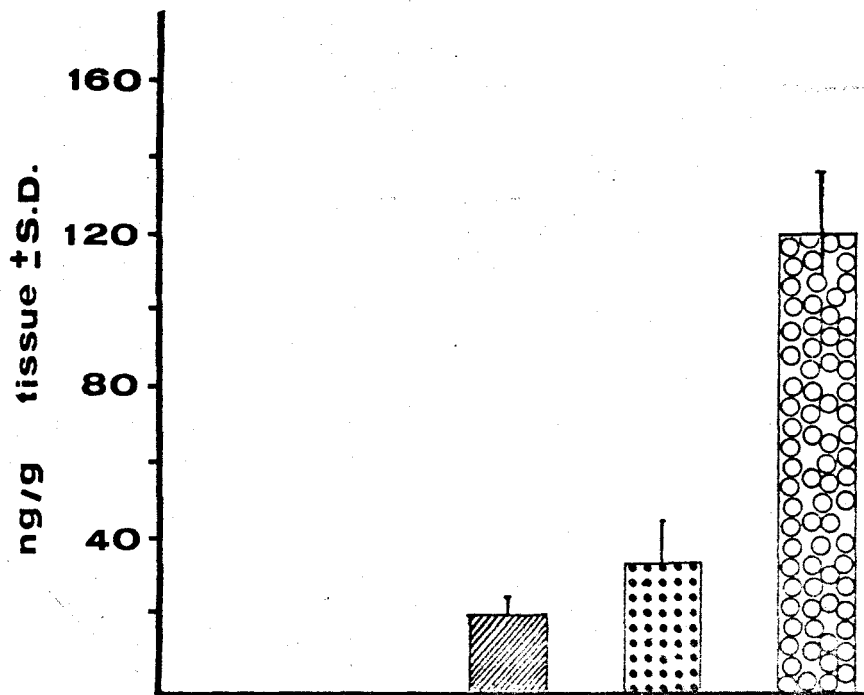


Fig. 15 Salsolinol concentrations in the combined brain parts of rats following acute treatment with:



pyrogallol/ethanol



DOPA/pyrogallol/ethanol



pargyline/pyrogallol/ethanol

in other experiments. Acute ethanol administration in pargyline/pyrogallol-treated rats again led to significant depletion of CAs from the brain.

#### b. EC/GC Analysis of Adrenal Glands

EC/GC analysis of the adrenal glands of rats treated with pargyline followed by pyrogallol/ethanol administration showed the formation of the new catechol compound, gigantanol. The concentration of this TIQ in adrenal glands of these experimental animals (Table 12) was not significantly higher than its concentration in the pyrogallol/ethanol adrenals (Table 10). Pargyline administration also did not lead to a significant change in the CA concentrations in the adrenal gland (Table 12 vs. Table 10). Unlike brain (Table 11) acute ethanol metabolism did not deplete the adrenal CAs in pargyline/pyrogallol rats (Table 12).

### III. FOLLOWING DOPA/PYROGALLOL PRETREATMENT

#### a. EC/GC Analysis of Combined Brain Parts

The quantitative determination of CAs in brain parts was performed on 5% GE XF-1105 and the results are shown in Table 13. Agreeing with earlier pyrogallol experiment #1 and #2, Table 9, ethanol decreased brain DA and NE very significantly. However, DOPA administration to the pyrogallol-treated animals before and during ethanol metabolism did not prevent the depleting effect of ethanol.

Using 3% OV-17 column, salsolinol was observed in the brain parts of rats treated with DOPA/pyrogallol/ethanol (Table 13). The level of endogenously formed salsolinol was estimated to be 193% of the amount formed in the brain parts of pyrogallol/ethanol treated rats,

Table 12

The effect of acute ethanol on gigantanol formation and the CA concentrations<sup>1</sup> in the adrenal glands of pargyline/pyrogallol-treated rats

Drug Treatment <sup>2</sup>	ug/g tissue $\pm$ S.D.			Gigantanol <sup>3</sup>
	DA	NE	E	
Pargyline-pyrogallol/ saline (4)	11.7 $\pm$ 3.0	172 $\pm$ 16	390 $\pm$ 95	not detectable
Pargyline-pyrogallol/ ethanol (7)	13.7 $\pm$ 3.5	164 $\pm$ 5.5	327 $\pm$ 55	4.0 $\pm$ 1.0

1. The values are corrected for recoveries based on <sup>14</sup>C-E recovery (range, 66.5% - 74.0%)

2. Number of rats/group in parenthesis

3. This figure at best should be considered a minimum estimate, because the aqueous E-AcH condensation itself forms 30-40% of supposedly other gigantanol isomers (1) (which were not separable in GC of adrenals), and because the calibration curve used for estimation was actually based on quantity of starting amine (E) rather than on major product (gigantanol).

Table 13

The effect of acute ethanol or DOPA/ethanol on salsolinol formation and the CA concentrations<sup>1</sup> in combined brain parts<sup>2</sup> of pyrogallol-treated rats.

Drug Treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.			
	DA	NE	E	Salsolinol
Pyrogallol/saline (5)	2.06 $\pm$ 0.26	1.02 $\pm$ 0.15	0.06 $\pm$ 0.01	not detectable
Pyrogallol/ethanol (7)	1.06 $\pm$ 0.10*	0.61 $\pm$ 0.07*	0.08 $\pm$ 0.02	0.015 $\pm$ 0.003
DOPA/Pyrogallol/ethanol(6)	1.10 $\pm$ 0.26*	0.59 $\pm$ 0.08*	0.08 $\pm$ 0.01	0.029 $\pm$ 0.015*

1. The values are corrected for recoveries based on <sup>14</sup>C-DA recovery (range 39.8%-47.7%)

2. Combined brain stem, caudate nucleus and midbrain including hypothalamus.

3. Number of rats/group in parenthesis

\* p < 0.05 compared to pyrogallol/saline

although this increase was not statistically significant.

#### b. EC/GC Analysis of Adrenal Glands

The level of E in the adrenal glands of pyrogallol treated rats was decreased by ethanol similar to previous pyrogallol experiments (table 12), but 1-DOPA given once before and once during ethanol metabolism, did not prevent ethanol depleting effect on E. Further, it should be noted that DOPA/Ethanol treatment significantly lowers both adrenal NE and DA in pyrogallol rats.

Administration of 1-DOPA did not have a significant effect on the concentration of the biosynthesized E-derived TIQ (gigantanol) in the adrenal glands of pyrogallol/ethanol treated rats (Table 14).

#### IV. FOLLOWING RESERPINE/PYROGALLOL PRETREATMENT

Rats treated with reserpine (2.5 mg/Kg i.p.) could not tolerate the following day's acute pyrogallol/ethanol treatment. All seven experimental rats died within 15-45 minutes after the first ethanol injection. The intention of this experiment was to deplete the tissue CAs and to observe the effect on TIQ biosynthesis. The experiment was not repeated with a lower dose of reserpine although such a change might be adequate to ensure survival.

#### V. IN THE ABSENCE OF DRUG TREATMENT

##### a. EC/GC analysis of whole brain

The qualitative EC/GC analysis of the brains of acute (7 hr) ethanol-treated rats with no drug pre-treatment was performed on 3% OV-17 (chromatogram is not shown). In the ethanol animals there was no detectable evidence for salsolinol biosynthesis in whole brain; CA-

Table 14

The effect of acute ethanol or DOPA/ethanol on gigantanol formation and the CA concentrations<sup>1</sup> in the adrenal glands of pyrogallol-treated rats

Drug Treatment <sup>2</sup>	ug/g tissue $\pm$ S.D.			
	DA	NE	E	Gigantanol <sup>3</sup>
Pyrogallol/saline(4)	14.4 $\pm$ 4.0	190 $\pm$ 58	560 $\pm$ 18	not detectable
Pyrogallol/ethanol(5)	14.0 $\pm$ 4.9	119 $\pm$ 48	301 $\pm$ 62*	3.7 $\pm$ 0.7
DOPA/Pyrogallol/ethanol(6)	8.0 $\pm$ 3.4**	100 $\pm$ 23*	216 $\pm$ 64*	3.3 $\pm$ 1.0

1. The values are corrected for recoveries based on <sup>14</sup>C-E recovery (range, 57.4%- 71.7%)

2. Number of rats/group in parenthesis

3. This figure at best should be considered a minimum estimate, because the aqueous E-AcH condensation itself forms 30-40% of supposedly other gigantanol isomers (1) (which were not separable in GC of adrenals), and because the calibration curve used for estimation was actually based on quantity of starting amine (E) rather than of major product (gigantanol)

\* p < 0.01 compared to pyrogallol/saline.

\*\* p < 0.05



rich brain parts were not examined separately in these experiments.

Again, similar to the preceding experiments with pyrogallol/saline and combined brain parts (vide supra), the whole rat brains from controls (saline only) showed the presence of significant quantities of the unknown LRT component. The acute ethanol treatment resulted in the almost complete disappearance of the LRT component from the whole brain, analogous to the pyrogallol/ethanol experiments.

The quantitative effect of ethanol on CA concentration of brain, evaluated on 5% GE XF-1105, is in table 15. A small and non-significant decrease of CAs was observed in the whole brain of ethanol-treated rats compared to saline controls.

#### G. CHRONIC ETHANOL METABOLISM IN THE ABSENCE OF DRUGS

##### I. GASTRIC INTUBATION TECHNIQUE

In this experiment, rats were intubated two and later three times daily for 15 days with 25% ethanol in water (v/v) or 21% dextrose in water (w/v) (Experimental, vide supra). Throughout the entire study the experimental animals were sluggish but did not show any other gross behavioral changes. The first day the food intake for experimentals dropped more than 90% of the pre-ethanol food levels, but then it gradually increased and stabilized at about 25% of this pre-ethanol level after five days. The pair-fed control rats showed hunger-related anxiety for the daily pellet ration, which had been determined from the experimental rat pellet consumption of the preceding day. Water intake ad lib did not differ significantly between controls and experimentals. In Fig. 16 are plotted food intake (experimental=controls) and body weight changes vs. time.

Table 15  
The effect of acute ethanol on the DA and NE concentrations <sup>1</sup> in the whole brain of rats

Experiment <sup>2</sup>	ug/g tissue $\pm$ S.D.	
	DA	NE
Saline (control) (4)	0.74 $\pm$ 0.15	0.38 $\pm$ 0.07
Ethanol (5)	0.61 $\pm$ 0.19	0.33 $\pm$ 0.07

1. The values are corrected based on the average recovery of <sup>14</sup>C-DA in these studies (44.4%)

2. Number of rats/group in parenthesis.

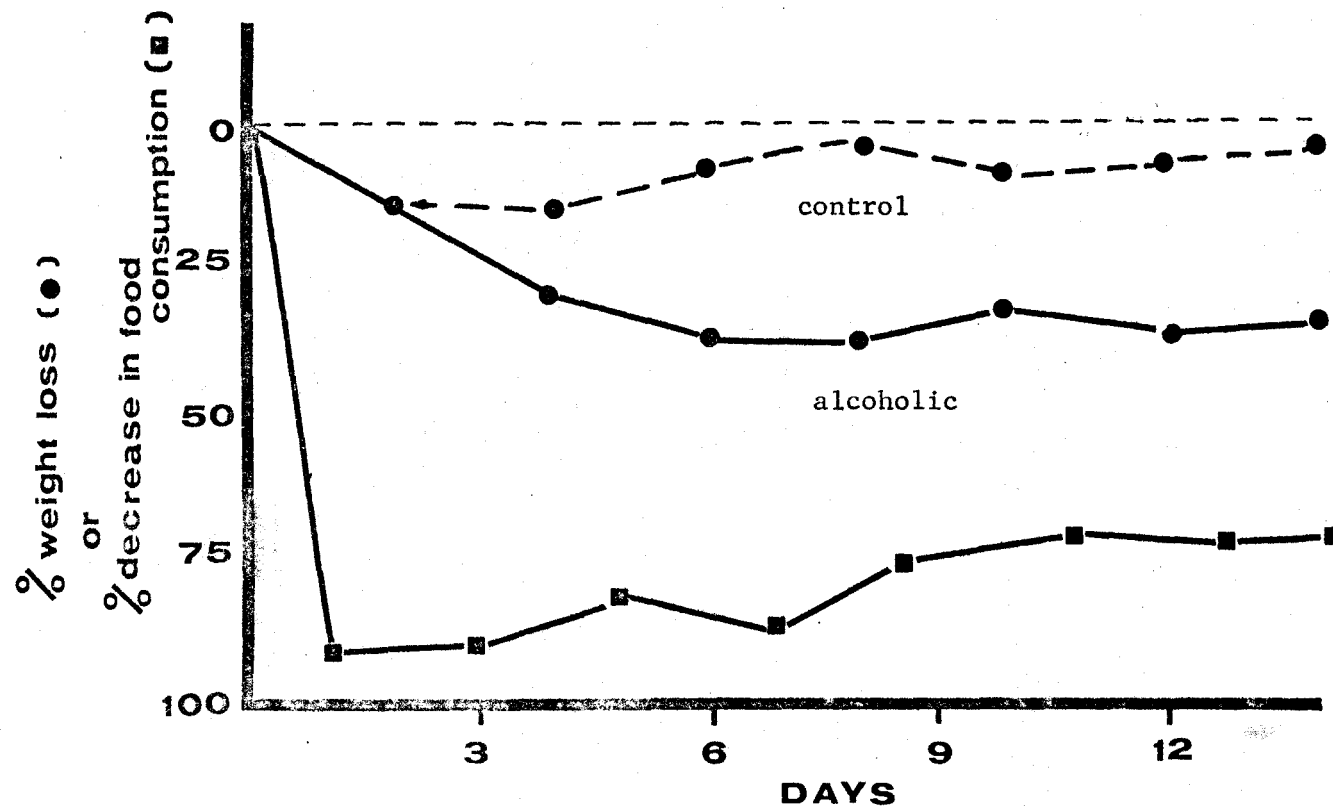


Fig. 16 The pattern of weight loss (circles) in 350 g rats following 15 days intubation with 25% ethanol (—●—) or 21% dextrose (---●---). The pattern of food consumption for either group is shown by (—■—).

#### a. EC/GC Analysis of Brain and Adrenal CAs

Fig. 17 shows the superimposed chromatogram of control (solid peaks) and experimental brain parts (dashed peaks). Evaluation of experimental brain parts showed no evidence for the occurrence of DA-derived salsolinol during chronic intoxication. However, similar to the saline controls in acute ethanol experiments, the brain parts of these control rats (pair-fed, isocaloric dextrose -treated) showed the presence of the LRT component (peak at 62 min) in large quantities. It was nearly absent from the brain parts of chronic ethanol rats (dashed line at 62 min).

In terms of potential TIQ biosynthesis, there was no EC/GC evidence on 5% GE XF-1105 for any new catechol compounds in the experimental adrenals. The superimposed chromatogram in Fig.18 shows the quantitative changes in adrenals between controls (solid peaks) and experimentals (dashed peaks).

The quantitative analyses of brain parts and adrenal glands are summarized in Table 16.

DA in the combined brain parts was decreased 24% by chronic ethanol and was unchanged in the adrenals. NE was lowered 43% in brain parts, and 54% in the adrenals, by the chronic ethanol metabolism. E was depleted 42% in the adrenals by ethanol.

#### b. EC/GC Estimation of O-methylated CA Derivatives in Brain

HFB-derivatives of the O-methylated CA metabolites extracted from the combined brain parts of each chronic ethanol and control rat were determined on 5% GE XF-1105. The results (Table 17) demonstrate a 130% increase in the level of the DA metabolite, 3-methoxy-tyramine, in the combined brain parts of alcoholic rats compared to controls.

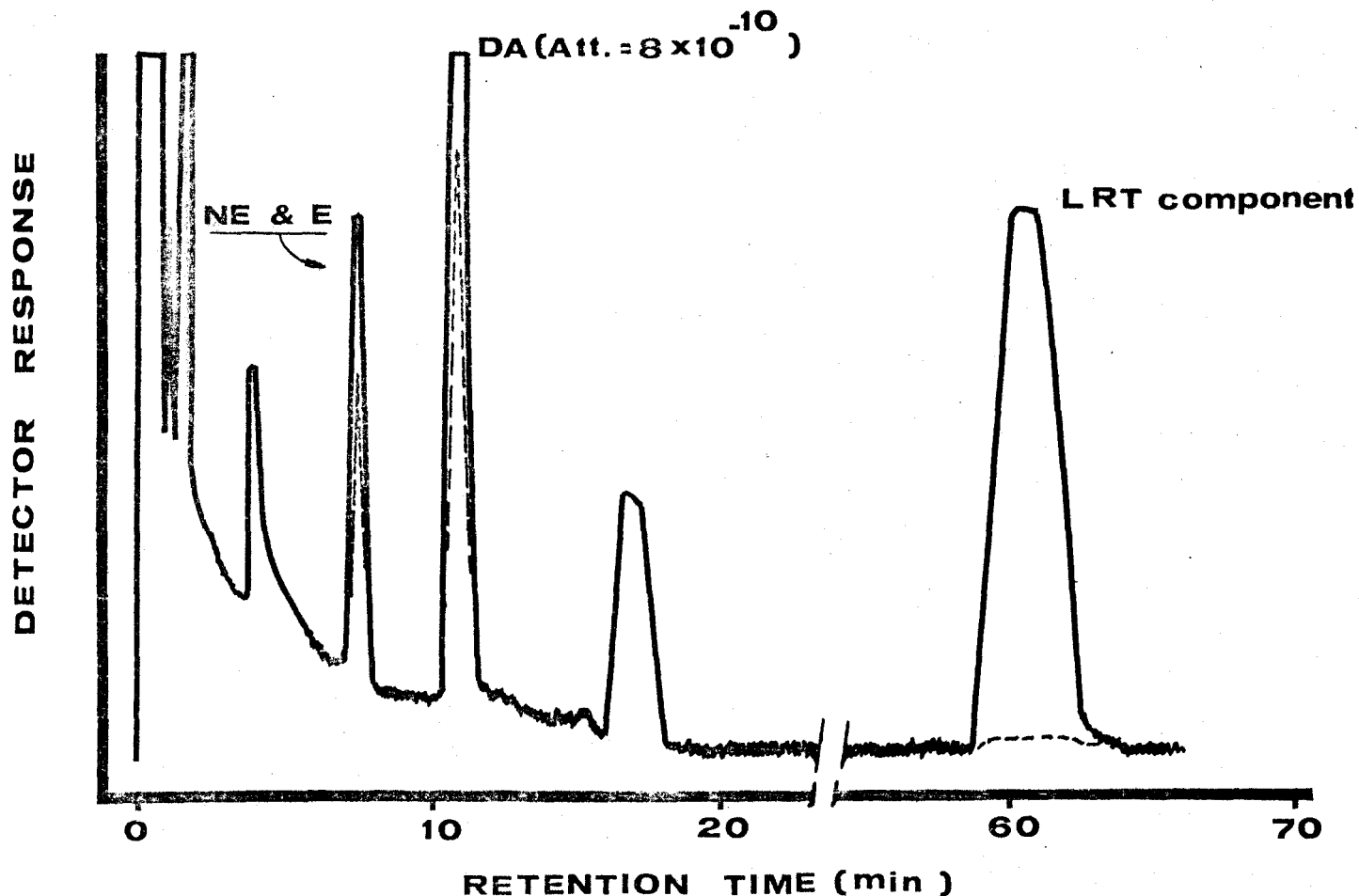


Fig. 17. Superimposed EC/GC chromatograms showing relative changes in HFB-catechol-containing compounds in the combined brain parts of rats following 15 days of chronic ethanol ( ---- ) or isocaloric dextrose intubation ( — ).

GC condition 3% OV-17 on 80/100 Gas Chrom Q , Column Temp. =  $160^{\circ}$  C;  $N_2$  flow rate 30 ml/min  
Att. =  $4 \times 10^{-10}$  AFS

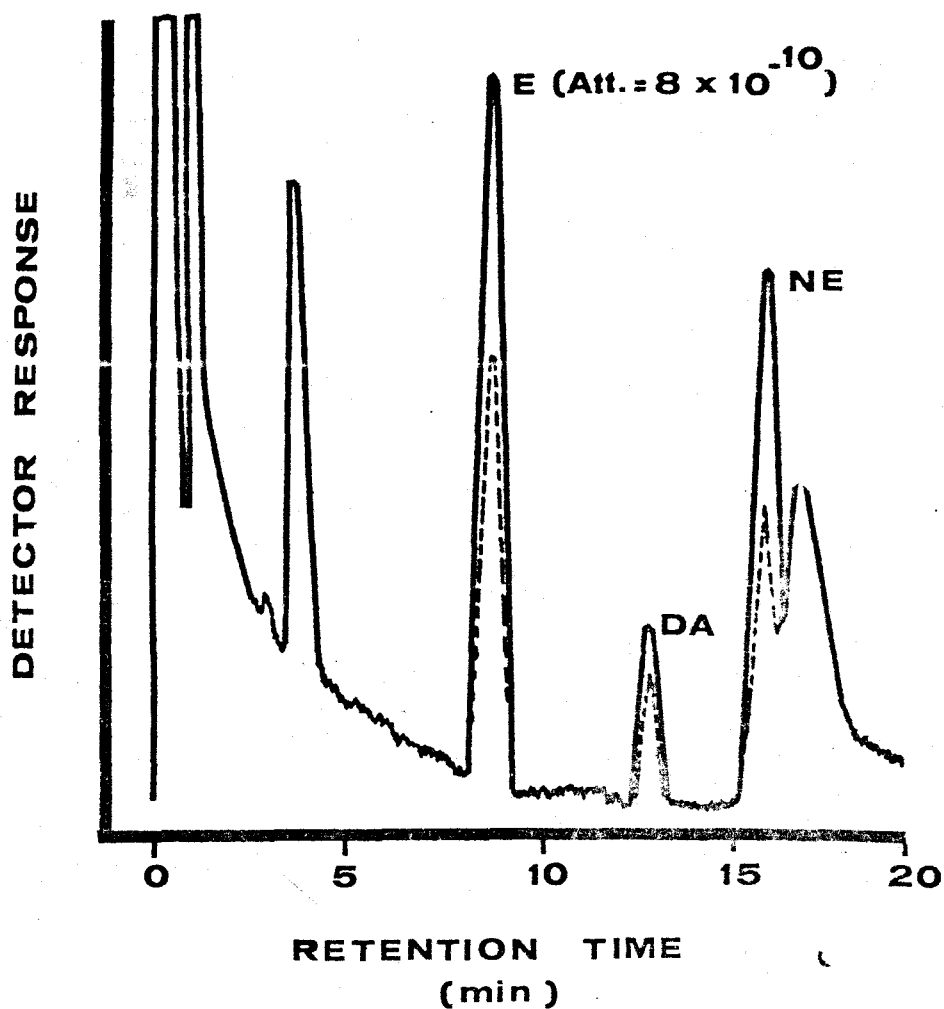


Fig. 18. Superimposed EC/GC chromatogram showing representatives changes in the HFB-catechol containing compounds in the adrenals of rats following 15 days of chronic ethanol (----) or isocaloric dextrose intubation ( — )

GC conditions: 5% GE XF-1105 ; Column Temp. = 175°C; N<sub>2</sub>, 35 ml/min on Gas Chrom Q; Att. =  $4 \times 10^{-10}$  AFS

Table 16

The effect of chronic ethanol on the CA concentrations in the combined brain parts<sup>1</sup> and adrenal glands of rats

CA	ug/g brain <sup>2</sup> $\pm$ S.D.		ug/g adrenals <sup>3</sup> $\pm$ S.D.	
	control (4)*	chronic ethanol (7)	control (4)	chronic ethanol (7)
DA	0.93 $\pm$ 0.10	0.68 $\pm$ 0.05**	21.3 $\pm$ 3.0	17.8 $\pm$ 3.6
NE	0.53 $\pm$ 0.08	0.30 $\pm$ 0.02**	192 $\pm$ 45	89 $\pm$ 16**
E			550 $\pm$ 91	344 $\pm$ 36**

1. Combined brain stem, caudate nucleus and midbrain, including hypothalamus.

2. The values are corrected based on the average recovery of <sup>14</sup>C-DA in earlier experiments

3. The values are corrected for recoveries based on <sup>14</sup>C-E recovery (range 65.3%-70.6%)

\* Number of rats/group

\*\* p < 0.01 compared to control

Table 17

The effect of chronic ethanol on 3-methoxy-tyramine and normetanephrine concentrations in the combined brain parts<sup>1</sup> of rats.

Drug Treatment <sup>2</sup>	ug/g tissue $\pm$ S.D.	
	3-methoxy-tyramine	normetanephrine
Dextrose solution (4)	244 $\pm$ 34.4	126 $\pm$ 8.6
Ethanol solution (7)	560 $\pm$ 75.0*	156 $\pm$ 13.3**

<sup>1</sup> Combined brain stem, caudate nucleus and midbrain including hypothalamus

<sup>2</sup> Number of rats/group in parenthesis

\*  $p < 0.01$  compared to dextrose control

\*\*  $p < 0.05$  compared to dextrose control



The increase in NE metabolite, normetanephrine, was smaller (24%) but was still statistically significant.

## II. CHRONIC i.p. INJECTION TECHNIQUE

As stated in Experimental, this experiment was terminated due to animal deaths. After the first day the food intake of experimental rats diminished to less than 10% of their pre-ethanol levels and there was a corresponding rapid weight loss. After four days the experimental rats developed severe diarrhea and most died within the next several days. No amine analyses were carried out.

## H. ACUTE METHANOL METABOLISM

### I. FOLLOWING PYROGALLOL PRETREATMENT

In this experiment rats were treated with pyrogallol one hour before the acute methanol administration described in the Experimental section. The blood levels of methanol and HCHO were not measured in these experimental animals.

The EC/GC results from the qualitative analyses of brain parts on 5% GE XF-1105 demonstrated no detectable appearance of CA/HCHO derived TIQs; therefore the chromatograms are not shown. However, the LRT component was obvious in the brain parts of both control (pyrogallol/saline) and experimental (pyrogallol/methanol) rats; i.e. the methanol administration did not lead to any apparent decrease in the unknown LRT component in the brain.

Quantitative analyses of CAs in the brain gave different results from those analyses of acute ethanol metabolism experiments. The concentrations of DA and NE in the combined brain parts from pyrogallol/

methanol rats, slightly higher than the control, were not statistically different (Table 18); the slight increase in brain E was statistically significant.

Fig. 19 is an EC/GC chromatogram of adrenals. It shows the presence of a new catechol component (hereafter, for discussion purposes, referred to as 1-nor gigantanol) appearing in all pyrogallol-methanol rats but not in any of the controls, which could be potentiated by the addition of the HFB-derivative of the major E/HCHO-derived TIQ to the GC injection solution. A second unidentified component was also seen in 3 of the 5 experimental adrenal glands. Its retention time was in vicinity of CA-derived TIQs, but did not correspond with any of the available compounds.

Table 19 shows the effect of methanol on CA concentrations in the adrenal glands of rats treated with pyrogallol. Large and statistically significant depletions in adrenal E and NE (86% and 75%, respectively) occurred due to the methanol metabolism in pyrogallol-treated rats.

## II. IN THE ABSENCE OF DRUG TREATMENT

As is apparent in the chromatogram, Fig. 20, acute methanol administration to the rats without prior drug treatment resulted in the formation of two new catechol compounds in adrenal glands. The first new peak corresponded to 1-nor-gigantanol, the cyclization product of E and HCHO. The height of this first new peak in the experimentals was increased when the HFB-derivative of E/HCHO cyclization products were added to the injection solution. The concentration of suspected 1-nor-gigantanol, when peak areas were compared, was 20-30% less than in adrenals of pyrogallol/methanol

Table 18

The effect of acute methanol on the CA concentrations<sup>1</sup> in the combined brain parts<sup>2</sup> of pyrogallol-treated rats

Drug treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.		
	DA	NE	E
Pyrogallol/saline (4)	1.92 $\pm$ 0.21	0.80 $\pm$ 0.09	0.066 $\pm$ 0.008
Pyrogallol/methanol (5)	2.10 $\pm$ 0.24	1.02 $\pm$ 0.18*	0.094 $\pm$ 0.010*

1. The values are corrected for recoveries based on <sup>14</sup>C-DA recovery (range, 41.3% - 49.9%).

2. Combined brain stem, caudate nucleus and midbrain, including hypothalamus.

3. Number of rats/group in parenthesis

\* p < 0.05 compared to pyrogallol/saline control

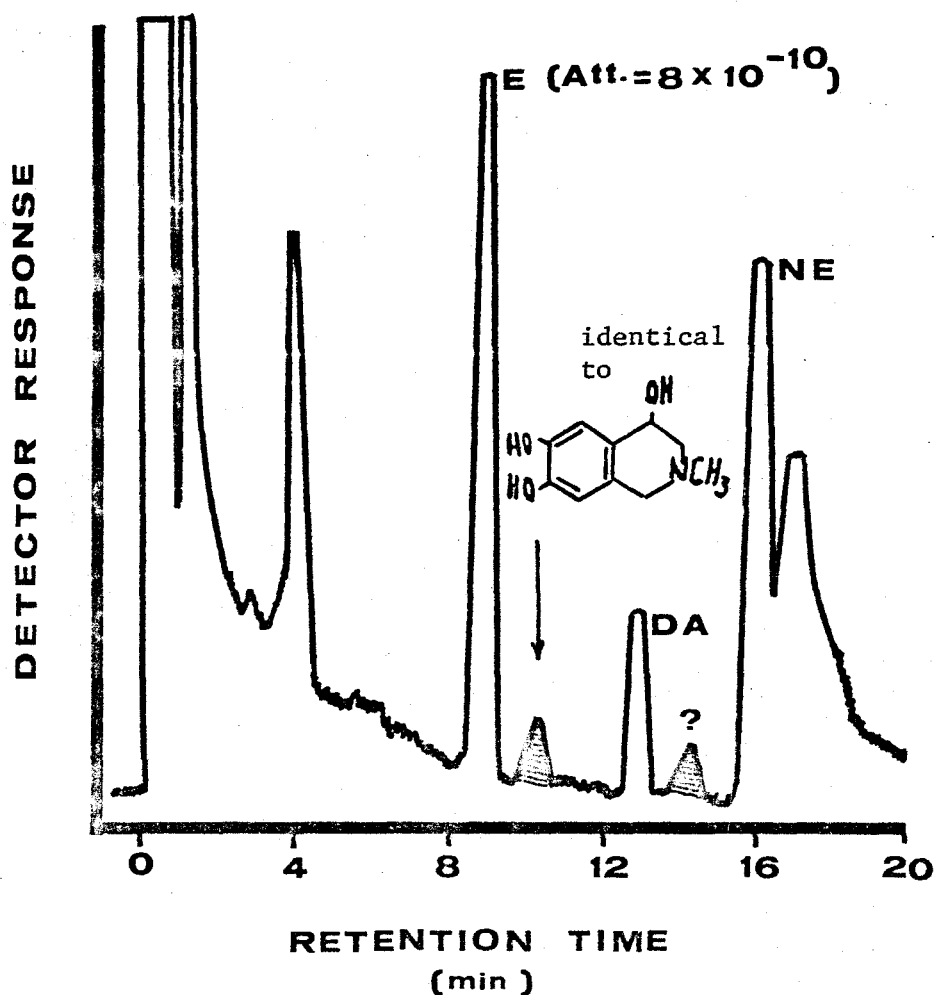


Fig. 19. GC chromatogram of HFB-catechol containing compounds in the adrenals glands of acute methanol intoxicated, pyrogallol-treated rats. Hatched peaks are absent from the adrenals of saline/pyrogallol-treated control rats.

GC conditions: 5% GE XF-1105 on 80/100 Gas Chrom Q, Column Temp. 175°C,  $N_2$ , 35 ml/min; Att=  $4 \times 10^{-10}$  AFS.

Table 19

The effect of acute methanol on CA concentrations<sup>1</sup> in the adrenal glands of pyrogallol-treated rats<sup>2</sup>

Drug treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.		
	DA	NE	E
pyrogallol/saline (4)	10.4 $\pm$ 2.0	92.6 $\pm$ 2.2	325 $\pm$ 17.0
pyrogallol/methanol (5)	8.2 $\pm$ 2.0*	22.7 $\pm$ 3.2*	87 $\pm$ 7.0*

1. The values are corrected for recoveries based on <sup>14</sup>C-E recovery (range, 61.1%-73.5%)

2. Rats weighed 550 $\pm$ 30g in this experiment.

3. Number of rats/group in parenthesis.

\*  $p < 0.01$

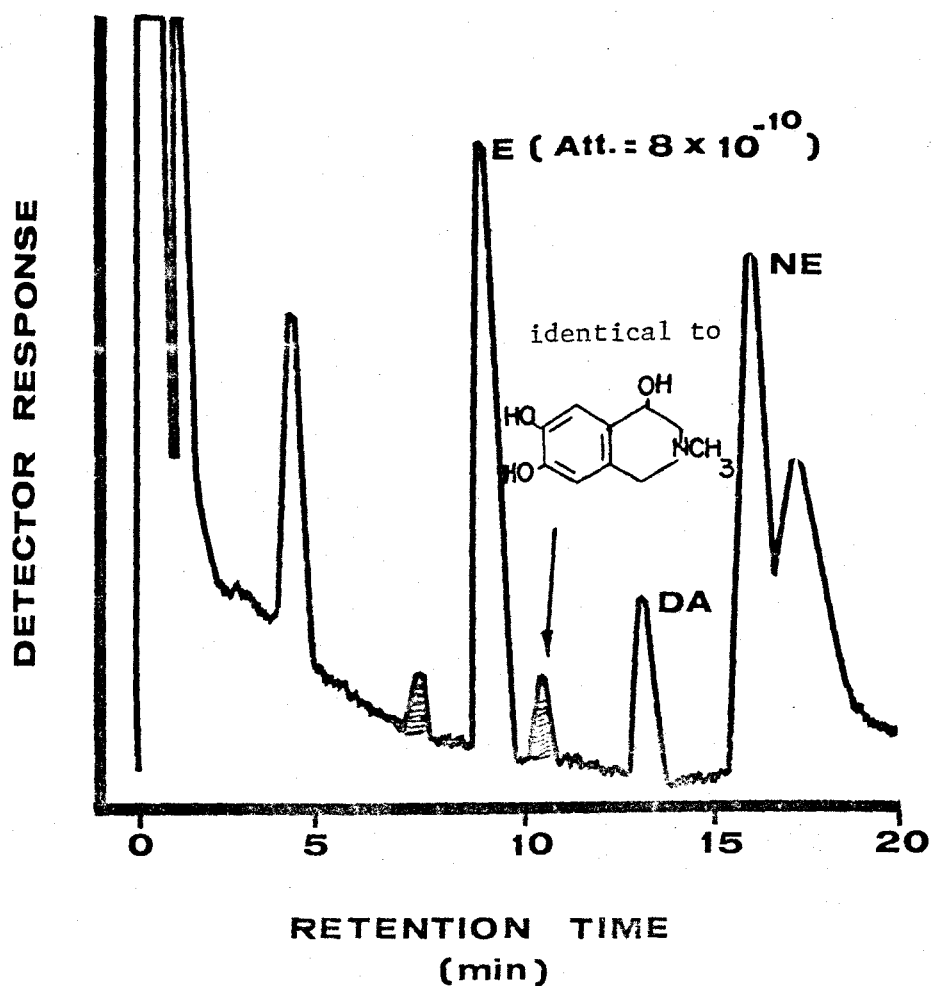


Fig. 20. EC/GC chromatogram of HFB-catechol containing compounds in the adrenals of acute methanol-intoxicated rats. Hatched peaks are absent from the adrenals of saline control rats.

EC/GC conditions: 5% GE XF-1105 on 80/100 Gas Chrom Q.  $T_c = 175^\circ\text{C}$   
 Att. =  $4 \times 10^{-10}$  AFS

rats (vide supra).

A second newly-formed component also absent from control adrenals, was observed in all adrenal glands of experimental rats at 7.50 min on the chromatogram (Fig.20). This unidentified component had different retention time than the second unidentified component (14.0 min) in the adrenal glands of pyrogallol/methanol treated rats. Although its retention time was in the range of simple TIQs, it did not correspond to the TIQ substrates available for this study.

In whole brain the qualitative results of acute methanol metabolism were approximately the same as in pyrogallol/acute methanol metabolism; that is, TIQ formation was not seen in the whole brain of methanol-treated rats. The LRT component was present in all brains and did not appear to be depleted by methanol metabolism.

The quantitative effects of methanol on CA concentrations in whole brain and adrenal glands of rats are in Table 20 and 21, respectively. Similar to its effect in the pyrogallol experiment, methanol did not alter the brain CA concentrations after seven hours metabolism, but it significantly decreased adrenal E (by 68%), adrenal NE (by 74%) and adrenal DA (by 55%).

Table 20

The effect of acute methanol on DA and NE concentrations<sup>1</sup> in the whole brain of rats

Drug treatment <sup>3</sup>	ug/g tissue $\pm$ S.D.	
	DA	NE
Saline (5)	0.55 $\pm$ 0.01	0.13 $\pm$ 0.01
Methanol (5)	0.48 $\pm$ 0.07	0.12 $\pm$ 0.01

1. The values are corrected for recoveries based on <sup>14</sup>C-DA recovery (range, 34.4%-48.2%)
2. Number of rats/group in parenthesis.
3. Number of rats/group in parenthesis



Table 21

The effect of methanol on CA concentrations<sup>1</sup> in the adrenal glands of rats

Drug treatment <sup>2</sup>	ug/g tissue $\pm$ S.D.		
	DA	NE	E
Saline (5)	13.6 $\pm$ 0.9	120 $\pm$ 4.8	512 $\pm$ 59
Methanol (5)	6.1 $\pm$ 1.6*	31 $\pm$ 4.1*	164 $\pm$ 44*

1. The values are corrected based on the average recovery of the other experiments.

2. Number of rats/group in parenthesis

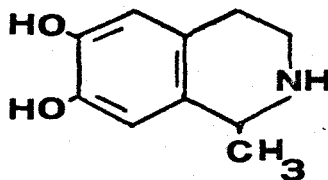
\*  $p < 0.01$  compared to control

## PART FOUR

### DISCUSSION AND CONCLUSIONS

#### A. TIQ BIOSYNTHESIS DURING ETHANOL METABOLISM

In three sequential experiments, each GC chromatogram (Fig.12 B) obtained from the combined brain parts of rats treated with pyrogallol followed by ethanol demonstrated a new component not observable in the pyrogallol/saline controls (Fig. 12 A) that had a retention time identical to salsolinol, the TIQ cyclization product of DA and AcD.



**SALSOLINOL**

The amount of this component was augmented when the HFB-derivative of authentic salsolinol was added to the injection solution (Fig.12 C). The possibility exists that the suspected salsolinol formed in the workup was eliminated by control homogenization with added AcD. Other catechol compounds such as N-acetyl-DA, epinine, dihydroxy-phenyl-ethanol, dihydroxyphenylglycol, as well as pyrogallol and its metabolites (3-methoxy-catechol and 2,3-dimethoxyphenol), all had retention times different from salsolinol and the new brain compound.

When brain DA was significantly elevated by pargyline administration to the animal prior to pyrogallol/ethanol treatment, the

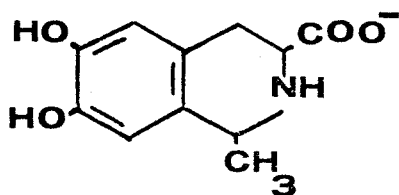
concentration of salsolinol in brain parts also was potentiated above the level observed in non-pargyline, pyrogallol/ethanol rats. Since precursor (DA) is increased due to MAO inhibition, higher levels of product (salsolinol in the high AcD environment occurred.

Using another derivatization method and column, salsolinol was again observed in combined brain parts of pyrogallol/ethanol rats. According to Wilk and Zimmerberg (99) and others (80, 106), identification of an unknown compound by GC is established through comparison of the retention times of two different derivatives of that compound on two or more columns with those of "authentic" substrate. Both of these techniques - varying the derivative (HFB-and PFP-) and the columns (GE XF-1105, SE-54 and OV-17), including column temperatures - were done in order to positively identify salsolinol.

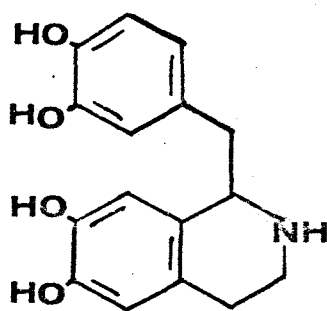
In the brain of rats receiving ethanol (acute or chronic) there was no sign of a new component which could be salsolinol. This no doubt was because of the 1/8 - 1/10 lower concentrations of AcD in the blood (1-3 ug/ml) and tissues of these rats than those treated with pyrogallol/ethanol. Pyrogallol administration is apparently the important factor in producing EC-detectable amounts of salsolinol in the tissues of ethanol-treated animals. Concerning pyrogallol's effect on AcD levels, it is likely that this comes about via inhibition of aldehyde oxidation pathways (105). Thus this drug potentiated the level of endogenously-formed catechol TIQ alkaloids not only by inhibiting the COMT catabolism (51, 52), but also by elevating the reactant, AcD (and possibly the CAs).

Nevertheless, these findings as well as the recent demonstration by Sandler et al (49) ought to be encouraging to investigators in the field of neurochemistry of alcoholism. However, the latter study only demonstrates the presence of TIQs in urine without proving that this formation was in blood or tissue. Since DA is a normal constituent of urine (100,101) and AcD is probably present in urine after ingestion of ethanol, it is probable that such a cyclization would take place in the urinary tract.

Unexpectedly, 1-DOPA injection before and during ethanol metabolism in pyrogallol-treated rats did not raise brain DA concentrations. Compared to pyrogallol/ethanol rats, only a slight but significant increase in salsolinol was observed. It has been shown that 1-DOPA raises brain DA in ethanol-intoxicated rats (109). One partial explanation is that condensation of 1-DOPA with AcD, present in abnormally high levels, forms another TIQ (3-carboxy-TIQ) and diverts 1-DOPA from its DA precursor role. The ease of this DOPA/AcD cyclization has been described by Brossi et al (43).



1-methyl-3-carboxy  
6,7-dihydroxy-TIQ  
(3-carboxy-TIQ)



Tetrahydropapaveroline (THP)

The postulation of in vivo aldehyde condensation with DOPA is not without precedent. Pfeiffer and Ebadi (118) have suggested that a similar cyclization between DOPA and pyridoxal phosphate takes place and leads to an inhibition of the DOPA-induced elevation of rat brain DA when the two compounds are administered together. However, this amino acid/AcH cyclization would have to be quantitatively significant in order to nullify the DA-elevating effect of DOPA. Alternatively, the 3-carboxy-TIQ itself may have inhibiting effects on DA biosynthesis or storage. According to Cohen (103), 3-carboxy-TIQs are not decarboxylated substantially by DOPA decarboxylase. This would explain the relative lack of change in the salsolinol concentrations, if DOPA/AcD condensation had occurred.

THP, a more complex DA-derived TIQ alkaloid (above), is reported to be found in rat brain homogenate incubated with DA and ethanol or AcD (104), and in the urine of Parkinsonian patients (49). THP was not detected in the brain parts of pargyline/pyrogallol/ethanol rats. However, the very low EC sensitivity of HFB-THP (MDQ=200 pg) would require that 100-200 fold more THP than salsolinol be biosynthesized in brain. Thus THP tissue biosynthesis could have occurred to a much greater extent than salsolinol biosynthesis in these experiments and still would be undetectable by the EC/GC method. Mass fragmentography would be more helpful than EC/GC for the detection of HFB-THP. The relatively high concentration of peripheral DA in Parkinsonian patients treated with l-DOPA no doubt was significant in facilitating the formation of THP in urine or tissue (49).

NE/AcD-derived TIQ was not observed in brain of experimental

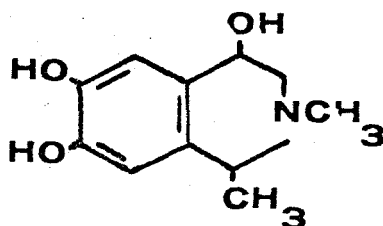
rats either. Several reasons may account for this. Fig. 8a shows clearly that the EC detector response to this TIQ is about 10-15 times less than to salsolinol. Furthermore, the velocity of reaction between NE and AcD is about 1/8th that of the DA and AcD reaction. Robbins (47) found the second order rate constant ( $\text{liter mol}^{-1} \text{min}^{-1}$ ) for alkaloid formation (pH 7.4 at 37°C) between AcH and DA, 1-DOPA or NE to be 15.3, 6.1 or 1.9 respectively. Finally brain NE concentration is over 50% less than DA.

As stated previously, ethanol metabolism in the "chronic alcoholic" rat did not result in detectable amounts of brain salsolinol. However, one major and possibly important difference was obvious between the GC chromatograms of control rat brain tissues and the chromatograms of brain tissue from chronic alcoholic as well as acute alcohol-treated rats (Fig.17 ). This was the existence of relatively large amounts of long retention time (LRT) component only in the controls. The identity of this component which is so rapidly depleted by ethanol remains undetermined, since this study was concerned primarily with TIQ biosynthesis. As a suggestion, the LRT component may be a volatile derivative of cofactor, NAD, which is depleted during ethanol metabolism. Consistent with this is the result that methanol, which is thought to be metabolized in the rat via hepatic catalase (107), did not lead to the disappearance of this unknown peak. However, the low activity of NAD-dependent aldehyde dehydrogenase in brain, as reported by Raskin and Sokoloff (108), does not support this suggestion.

Polyhydroxy steroids, when derivatized with HFB-groups, may have long retention times. The LRT component may be such a normal brain steroid which is depleted by ethanol or its metabolites or by some process

activated by ethanol, such as CNS transport (141). No EC/GC comparisons were done of retention times of HFB-derivatized NAD or any other normal brain material to the LRT component in normal rat brain.

TIQ biosynthesis in adrenal glands of pyrogallol/ethanol rats was also observed. The experimental animals demonstrated the presence of a new adrenal catechol derivative which was absent in pyrogallol/saline controls. This component could be augmented by the addition to the injection solution of the HFB-derivative of gigantanol (1,2-dimethyl-4,6,7-trihydroxy-TIQ).



**GIGANTINOL**

Pargyline pretreatment did not affect the quantity of biosynthesized gigantanol in the adrenal glands. The reason that this drug acted differently between brain and adrenal gland is not clear but may involve the possible specific effects of pargyline on different MAO isoenzymes (110,111,112,113). Selective inhibition of MAO action on different substrates has been reported by several investigators (112,114). Zeller (115) noted the differences in potency of pargyline as an MAO inhibitor, depending on which substituted benzylamine was used as a substrate. Squires (116), studying the selective action of various inhibitors on MAO in different organs of animals, suggested the presence of two MAO

fractions, A and B, in varying ratios in different tissues. It was shown that pargyline selectively inhibits the B fraction (116) of MAO in most species. To our knowledge no investigation has been carried out on the types of MAO in the adrenal glands. It is possible that A/B ratio of MAO is much larger in adrenal glands than brain, therefore making MAO less sensitive to pargyline inhibition. Nevertheless, 90% inhibition of MAO enzyme apparently is required (117) to fully observe elevation in CA levels. These findings may describe in part why changes were not observed in adrenal CAs and gigantanol due to pargyline administration, while significant increases were observed in brain DA and salsolinol concentrations.

The effect of 1-DOPA on the level of E and gigantanol in adrenals of pyrogallol/ethanol rats was insignificant. As mentioned above in Discussion, it is possible that the normal metabolic pathway of DOPA-DA in the presence of high AcD concentration is diverted to the formation of 3-carboxy-TIQ. This TIQ might be exerting an inhibitory effect on CA metabolism at the decarboxylase step, or interfering with CA storage in chromaffin cells.

#### B. TIQ BIOSYNTHESIS DURING METHANOL METABOLISM

Acute alcohol intoxication experiments also were carried out with methanol. The metabolite, formaldehyde (HCHO) condenses with CAs in vitro 25-50 times faster than AcD (1), and therefore TIQ formation might be expected. Administration of methanol to untreated or pyrogallol-treated rats resulted in a new adrenal product with an EC/GC retention time agreeing with 1-norgigantanol (the simple TIQ derivative of E and HCHO). TLC evidence for adrenal norgigantanol and the NE/HCHO-derived TIQ has been reported (45). In these present exper-



iments there was no indication of the NE/HCHO derived alkaloid in adrenals.

Pyrogallol administration did not increase the concentration of suspected 1-norgigantanol in the adrenals. The level of blood HCHO in rats after treatment with methanol or pyrogallol/methanol was not determined. Apparently pyrogallol does not inhibit the catalase system (105) which is responsible for methanol metabolism in rat(107). Therefore, HCHO level, unlike AcD, would not be expected to be increased by pyrogallol, and detectable TIQ concentrations would depend only on the COMT inhibitory effect of pyrogallol.

A second new compound in the range of TIQ and CA retention times with a peak area about 25% less than that of 1-norgigantanol consistently was seen in the chromatograms of the adrenals from rats treated with methanol only (Fig. 20, page 81). The possibility exists that this was another oxidized catechol metabolite of 1-norgigantanol or the TIQ derived from NE, but due to the lack of authentic substrates, this idea could not be checked out. However, it is perhaps significant that pyrogallol pretreatment caused this second unidentified adrenal compound to disappear (or change) concomitant with the formation of another new "second" peak still within the range of TIQs and CAs at 14.0 min (Fig. 19, page 79).

It is possible that both new "second" peaks are E-derived TIQ products from two different catabolic pathways, one significant and the other normally insignificant. Pyrogallol may cause a metabolic shift towards the normally insignificant pathway. It is also possible that pyrogallol somehow affect the E/HCHO cyclization reaction leading to a different isomeric product.

An alternative explanation is that the new "second" adrenal peaks in methanol and pyrogallol/methanol rats are CA rather than TIQ metabolites, 3-methoxy-4-OH-phenylglycol (MHPG) and dihydroxy-phenylglycol (DHPG), respectively. MHPG, considered to be the major catabolite of brain NE (134, 139), may be a minor catabolite in the (control) adrenals. Methanol metabolism, by increasing the reductive capacity of adrenal tissue, might increase MHPG concentrations sufficiently so that MHPG would be carried over or extracted by the  $\text{Al}_2\text{O}_3$  procedure. Inhibition of COMT by pyrogallol would decrease MHPG and increase its precursor, DHPG, perhaps to detectable concentrations. Further work is necessary in order to identify these new components in the adrenals of methanol-intoxicated rats.

It is puzzling that, contrary to the adrenal situation, there was no GC evidence for TIQ biosynthesis from the condensation of brain CAs with HCHO in the methanol-intoxicated rats (with or without pyrogallol). Due to the high reactivity of HCHO with DA (119) it is possible that some kind of condensation reaction may have taken place but metabolism of the TIQ (1-norsalsolinol) occurred during the 8 hr experimental time. Time studies should be performed to follow up this suggestion.

#### C. CA Concentrations in Normal Rat brain and Adrenal Glands

It is known that HFB-derivatives of biogenic amines show larger E.C. responses than their TFA- and PFP-analogues (83, 123). In addition, because of longer retention times, HFB-derivatives sometimes demonstrate better GC resolutions. These aspects were used to separate HFB-TIQs from the HFB-CAs in pg amounts. Although the % recovery was lower

than the Arnold and Ford method (83), the reproducibility for catechol compounds and their metabolites was excellent. The application of  $^{14}\text{C}$ -tracers was used to determine total losses from tissue during the purification steps and corrections for this loss served for the precision of results. The precision obtained from repeat estimations of CAs in the brain parts of pyrogallol-treated rats (Table 22), demonstrates the reliability of this EC/GC technique in tissue analyses.

This technique was used successfully for the analysis of less than 10 mg of brain tissue and for the recovery of as little as 10 ng of added 4,6,7-trihydroxy-TIQ with tissue DA and NE. DA, NE and E concentrations were also estimated in the whole brains of individual rats and the results (Table 5, p 45) compared favorably with the spectrofluorometric analyses of Shellenberger and Gordon (97) and Barchas et al (96).

In this EC/GC technique, the small difference observed between the values of brain NE on two different columns (Table 5, p 45) could be due to the lack of separation of NE from E on SE-54 (or OV-17) as opposed to GE XF-1105.

The concentrations of CAs in adrenal glands of normal rats were found with HFB-derivatives on GE XF-1105 to be in close agreement with the GC results reported by Imai et al (95). Concentrations of adrenal E measured by spectrofluorometric methods are higher than that determined by GC methods (Table 6, p 46). This diversity could be due to the strain differences in experimental animals, or to interference of non-specific materials with the spectrofluorometric method.

Table 22

Comparison of the results obtained from repeat estimation of CAs in the combined brain parts of pyrogallol-treated rats<sup>1</sup>

Experiment No.	No. of rats	ug/g tissue <sup>2</sup> $\pm$ S.D		
		DA	NE	E
1	4	1.92 $\pm$ 0.21	0.80 $\pm$ 0.09	0.063 $\pm$ 0.009
2	6	1.98 $\pm$ 0.17	0.99 $\pm$ 0.11	0.080 $\pm$ 0.016
3	5	2.06 $\pm$ 0.26	1.02 $\pm$ 0.15	0.058 $\pm$ 0.010
4	5	2.02 $\pm$ 0.50	1.05 $\pm$ 0.31	

1 control rats for acute ethanol metabolism experiments, Experimental, p.32

2 the values are calculated per g combined brain parts (brain stem, caudate nucleus and midbrain, including hypothalamus)

The results from pyrogallol-treated rats indicate the existence of E in the rat brain. The presence of E in the CNS has been a matter of controversy. Since the suggestion was made by Von Euler (124) that E (sympathin) exists in mammalian brain, many investigations have been made on the problem. The ratio of E to the sum of E and NE in rat brain was reported to be 0.045 by Gunne (125) 0.034 by Montagu (126), determined by fluorometric methods. On the other hand, Sano and Taniguchi (127), using Amberlite IRC 50 for the separation of CAs, and the trihydroxyindole fluorometric method, found no E in the brain. Carlsson (128) and McGeer and McGeer (129) also failed to detect this amine by fluorometry. Anton and Sayre (89) conjectured that the large amount of DA in the brain may interfere with the fluorometric measurement of E. Imai et al (95) with EC/GC and TFA derivatives, have measured 150 ng E/ g of whole rat brain. As shown in Table 9, p 56 , the average concentration of E in the combined brain parts of pyrogallol-treated rats was  $66 \pm 8$  ng/g tissue, which is about 6.6% of the NE + E concentrations in the same brain sample. The possibility exists that circulating E in blood contributes to this "brain E" value. However, the small quantity of blood within the brain at decapitation with an E concentration of 60 ng/L ( 102) could not contribute more than 50-100 pg E to the average value.

E was not estimated in brain samples from non-pyrogallol-treated rats (control). One reason for this was that in the whole rat brain analysis a relatively large unknown peak tended to overlap with the retention time of HFB-E on GE XF-1105. It was for this reason, in fact, that organic solvent extraction (91), in case the interfering component

was lipid, was attempted in one experiment (Experimental, vide supra), but with little success. In combined brain parts this component interfering with E, although diminished, was still apparent. Pyrogallol pretreatment served to potentiate brain E (perhaps due to inhibition of COMT catabolism) and permit its estimation in saline controls.

D. THE EFFECT OF ALCOHOL METABOLISM ON RAT BRAIN AND ADRENAL CA CONCENTRATIONS

Conflicting data on the effect of ethanol on brain amine concentrations has been reported (Introduction, vide supra). This discrepancy has been attributed by some to ethanol-derived AcD, causing the release of CAs from the CNS(26,34). In this study, acute ethanol metabolism in the absence of other drugs did not change brain or adrenal CA concentrations, in agreement with Haggendal and Lindquist(31) and others (30). However, sharp decreases in the respective CAs to ca. 50% of control brain DA and NE and control adrenal E concentrations were observed when the acutely intoxicated rats were pretreated with pyrogallol. Because blood AcD levels during ethanol oxidation were elevated by pyrogallol, the decreases in CAs probably reflect in part or in total the releasing action of AcD on CA stores.

Chronic ethanol metabolism for 15 days (in the absence of other drugs), however, which generates an "alcoholic" rat(77), did result in significant 25-50% decreases in brain DA and NE and adrenal E. Thus when control rats are properly handled, and paired nutritionally with experimental alcoholic rats, changes in CA due to "alcoholism" become apparent. The lack of adequate nutritional controls may explain why some investigators reported no brain amine changes fol-

lowing chronic ethanol gavage in the rat (33). Furthermore, the depletion observed here may be due as much to the chronic presence of AcD (estimated at 4 ug/ml at sacrifice) as to ethanol.

Alternately, the data from methanol metabolism experiments indicate that HCHO-derived TIQ may be involved in the CA depletion process. In adrenals, which were shown to possibly contain TIQ(s) derived from E, significant depletion of E and NE was seen, whereas in the same rat, no TIQs and no CA decreases were observed in the brain.

The mechanism by which HCHO-derived TIQs might lower CA concentration is speculative. Cohen has shown that DA/HCHO cyclization products release and inhibit the uptake of CAs in vitro (55, 130). It is possible that certain TIQs can displace CAs from storage and receptor sites. Tabakoff et al (132) have reported that salsolinol and morphine easily displace 5-HT from its "binding site" in brain tissue. Perhaps TIQs act on membrane as has been suggested for 6-hydroxy-DA (131) to increase permeability to or block the uptake/storage of CAs. The striking structural similarity, shown in Fig.21, between a possible quinoid oxidation product of DA/HCHO-derived TIQ and the hypothesized active quinone from of the in vivo CA depletor, 6-hydroxy-DA (133) suggests a mechanism by which the TIQ itself could lower CA levels. Pharmacological work is necessary with the synthetic TIQ.

As DA and NE in brain during chronic ethanol consumption are decreased by whatever mechanism, one expects an increase in 3-methoxytyramine and normetanephrine, metabolites produced by the action of extracellular COMT on the amines. In fact, this proved to be the case. Brain parts of rats chronically treated with ethanol were analyzed to estimate the level of O-methylated metabolites of CAs. Substantial increases of 3-methoxytyramine (130%) and normetanephrine (24%) were

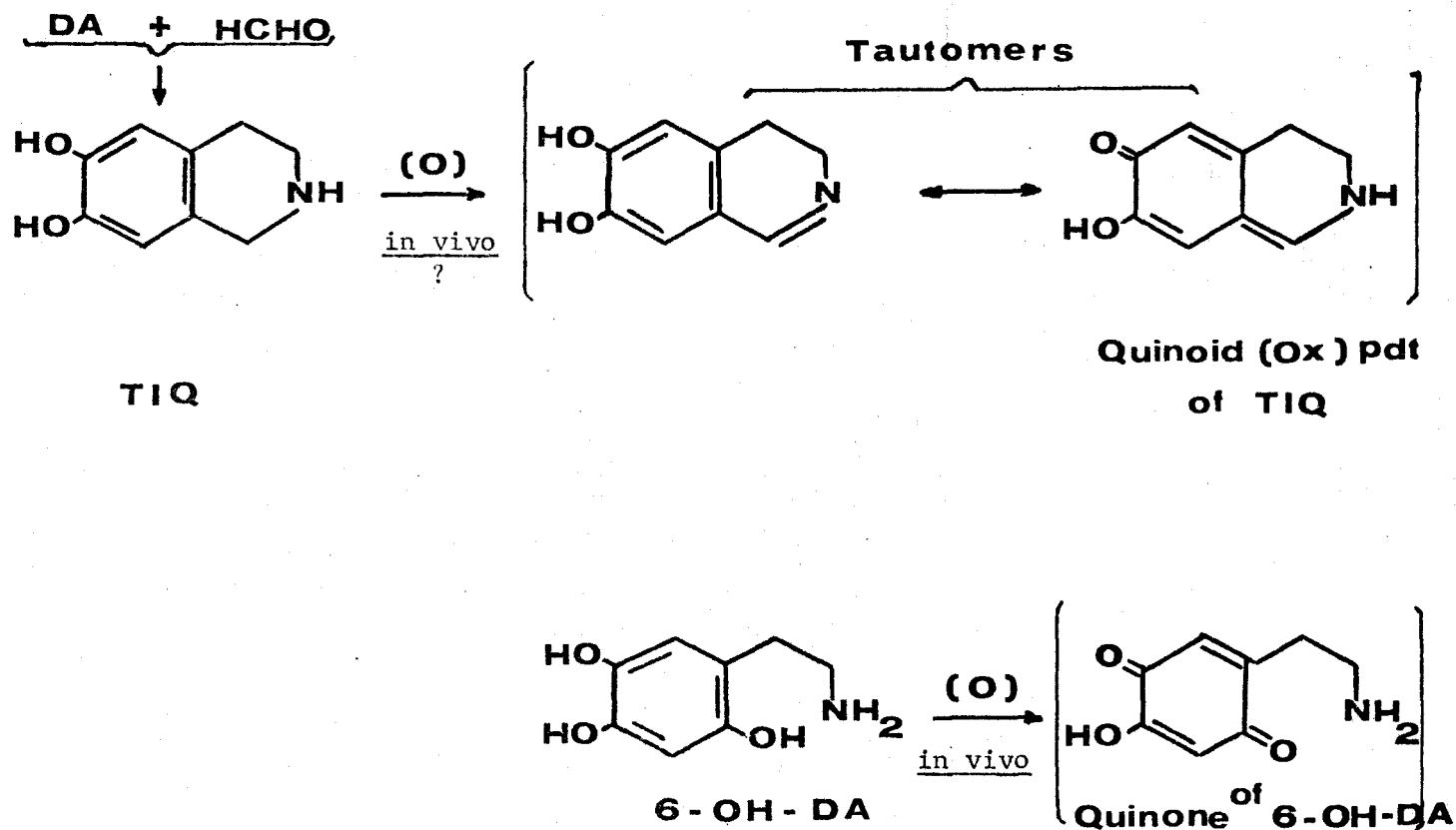


Fig. 21      Structural resemblance between a possible quinoid oxidation product of a TIQ and the oxidized quinone of 6-hydroxy-DA (6-OH-DA)



observed in the "alcoholic" rat brains compared to controls. It is known that DA is found in higher concentration in soluble fractions (136) of nerve cells compared to particulate fractions where NE is stored (120). Therefore DA would be more susceptible to the action of COMT and would be expected to be more extensively O-methylated than is NE.

## BIBLIOGRAPHY

1. G. Cohen and M.A. Collins, "Alkaloids from catecholamines in adrenal tissue, possible role in alcoholism," Science, 167: 1749-1751 (1970)
2. V.E. Davis and M.J. Walsh, "Alcohol, amine and alkaloids. A possible biochemical basis for alcohol addiction." Science, 167: 1005-1007 (1970)
3. N.E. Anden, A. Carlsson, N.A. Hillarp and T. Magnusson, "Noradrenaline release by nerve stimulation of the spinal cord," Life Sci., 4: 129-132 (1965)
4. N.A. Hillarp, K. Fuxe and A. Dahlstrom, "Demonstration and mapping of central nervous system containing dopamine, noradrenaline and 5-hydroxytryptamine and their reaction to psychopharmacia," Pharmacol. Rev., 13: 727-741 (1966)
5. I. Kopin, The Adrenergic synapse, in The Neurosciences, (G. Quarton, T. Melnechuk and F. Schmitt, eds.) pp. 427-432, Rockefeller U. Press, N.Y. (1967)
6. Lancet, Leading article, "Alcohol addiction: A biochemical approach." Lancet, ii, 24-25, (1972)
7. A.A. Smith and S. Gitlow, "Effect of disulfiram and ethanol on the catabolism of norepinephrine in man," in Biochemical Factors in alcoholism, (R.P. Maickel, ed.) pp. 53-59, Pergamon, New York, (1967)
8. V.E. Davis, H. Brown, J.A. Huff and J.L. Cashaw, "Ethanol-induced alteration of norepinephrine metabolism in man," J. Lab. Clin. Med., 69: 132-140 (1967)
9. V.E. Davis, H. Brown, J.A. Huff and J.L. Cashaw, "The alteration of serotonin metabolism to 5-hydroxytryptophol by ethanol ingestion in man." J. Lab. Clin. Med., 69: 132-140 (1967)
10. V.E. Davis and M.J. Walsh, "Effect of ethanol on neuroamine metabolism," in Biological Basis of Alcoholism (Y. Israel and J. Mardones, eds.), pp. 73-102, Wiley-Interscience, New York, (1971)
11. M. Tytell and R.D. Myers, "Metabolism of (<sup>14</sup>C)-serotonin in the caudate nucleus, hypothalamus and reticular formation of the rat after ethanol administration," Biochem. Pharmacol., 22: 361-372 (1973)
12. B. Tabakoff, "Alcohol and aldehyde metabolism in brain," Am. Chem. Soc., 166th national meeting 172-173 (1973)
13. G. Rosenfeld, "Inhibitory influence of ethanol on serotonin metabolism," Proc. Soc. Exp. Biol. Med., 103: 144-149 (1960)

14. A. Feldstein, H. Hoagland, K. Wong and H. Freeman, "Biogenic aldehydes and alcohol," Quart. J. Stud. Alc., 25:218-225 (1964)
15. R. A. Lahti and E. Majchrowicz, "The effect of acetaldehyde on serotonin metabolism," Life Sci., 6: 1399-1406, (1967)
16. D. Eccleston, W.H. Reading and I.M. Ritchie, "5-Hydroxytryptamine metabolism in brain and liver slices and the effect of ethanol," J. Neurochem., 16: 274-276 (1969)
17. R.A. Lahti and E. Majchrowicz, "Acetaldehyde-an inhibitor of the enzymatic oxidation of 5-hydroxyindoleacetaldehyde," Biochem. Pharmacol., 18: 535-538 (1969)
18. J.P. Von Wartburg and H. Aepli, "Der einfluss langdauernder athylalkoholbelastung auf die catecholaminausscheidung in harn der ratte," Helv. Med. Acta, 28: 89 (1961)
19. G.I. Klingman and M. Godall, "Urinary epinephrine and levarterenol excretion during acute sublethal alcohol intoxication in dogs," J. Pharm. Exp. Ther., 121: 313-318 (1957)
20. V.J. Schenker, B. Kissin, L.S. Maynard and A.C. Schenker, "The effect of ethanol on amine metabolism in alcoholism," in Biochemical Factors in Alcoholism, (R.P. Maickel, ed.), pp. 39-52, Pergamon, New York (1967)
21. J. H. Mendelson, M. Ogata, and N.K. Mello, "Catecholamines and cortisol in alcoholics during experimentally induced intoxication and withdrawal," Fed. Proc., 28: 262 (1969)
22. C. Carlson, and J. Haggendal, "Arterial noradrenaline level after ethanol withdrawal," Lancet, ii, 889 (1967)
23. M. Ogata, J.H. Mendelson, N.K. Mello and E. Majchrowicz, "Adrenal function and alcoholism: II. Catecholamines," in Recent Advances in Studies of Alcoholism, (M.K. Mello and J.H. Mendelson, eds.) pp. 140-172, U.S. Government Printing Office, Publ. No. (HSM) 71-9045, Washington, D.C. (1972).
24. E.S. Perman, "The effect of acetaldehyde on the secretion of adrenaline and noradrenaline from suprarenal gland of the cat," Acta. Physiol. Scand. 43: 71-76 (1958)
25. J. Akabane, S. Nakanishi, H. Kohei, S. Asakawa, R. Matsumura, H. Ogata, and T. Miyazawa, "Studies on sympathomimetic action of acetaldehyde: II. Secretory response of adrenal medulla to acetaldehyde: Experiments with the perfused cat adrenals," Japan J. Pharmacol. 15: 217 (1965)
26. M.J. Walsh and E.B. Truitt, "Release of 7-<sup>3</sup>H-norepinephrine in plasma and urine by acetaldehyde and ethanol in cat and rabbits," Fed.Proc., 27: 601 (1968)

27. D. Gursev, J.W. Wester and R.E. Olson, "Effect of ethanol administration upon serotonin and norepinephrine levels in rabbit brain," J. Clin. Invest., 38: 1008-1009 (1959)
28. D. Gursev and R.E. Olson, "Depression of serotonin and norepinephrine levels in brain stem of rabbit by ethanol," Proc. Soc. Exp. Biol. Med., 104: 280-281 (1960)
29. H. Corrodi, K. Fuxe and T. Hokfelt, "The effect of ethanol on the activity of central catecholamine neurons in rat brain," J. Pharm. Pharmacol., 18: 821-823 (1966).
30. D. H. Efron and G.L. Gessa, "Failure of ethanol and barbiturates to alter brain monoamine content," Arch. intern. Pharmacodyn., 142: 111-116 (1963)
31. J. Haggendal and M. Lindquist, "Ineffectiveness of ethanol on noradrenaline, dopamine and 5-hydroxytryptamine levels in brain," Acta Pharmacol. Toxicol., 18: 278-280 (1961)
32. G.R. Pscheidt and B. Issekutz Jr.; "Importance<sup>of</sup> acetaldehyde in the action of ethanol on brain stem concentration of biogenic amines," Quart. J. Stud. Alc., 22: 550-553 (1961)
33. H. Walgren, "Neurochemical aspects of tolerance to and dependence on ethanol," in Adv. Exp. Med. Biol., 35: 15-31 (1973)
34. G. Duritz and E.B. Truitt, Jr., "Importance of acetaldehyde in the action of ethanol on brain norepinephrine and 5-hydroxytryptamine," Biochem. Pharmacol., 15: 711-721 (1966)
35. M.J. Walsh, "Biogenesis of biologically active alkaloids from amines by alcohol and acetaldehyde," Ann. N.Y. Acad. Sci., 215: 98-110, (1973)
36. J.F. Berry and E. Stotz, "Acetylcholine and acetoin in brain during acetaldehyde intoxication," Quart. J. Stud. Alc., 17: 190-194 (1956)
37. W.W. Westerfeld, R.J. Bloom, L.M. Shaw and P.G. Fuller, "The acetaldehyde condensation reaction with  $\alpha$ -ketoglutarate," in Biochemical and Clinical Aspects of Alcohol Metabolism, (V.M. Sardesai, ed.) pp. 58-67, Charles C. Thomas, Springfield, Ill. (1969)
38. H.P.T. Ammon, C.J. Estler and F. Heim, "Inactivation of coenzyme A following the administration of ethanol in vivo," Biochem. Pharmacol. 18: 29-33 (1969)
39. R.A. Beck, C.C. Pfeiffer, V. Iliev and L. Goldstein; "Cortical EEG stimulant effect in the rabbit of acetaldehyde-biogenic amine reaction products," Proc. Soc. Exp. Biol. Med., 128: 823-826 (1968)

40. W.M. McIsaac, "Formation of 1-methyl-6-methoxy-1,2,3,4,-tetrahydro-2-carboline under physiological conditions," Biochem. Biophys. Acta, 52: 607-609 (1961)
41. R.G. Taborsky and W.M. McIsaac, "The synthesis and preliminary pharmacology of some 9-H-pyrido (3,4-b) indoles (B-carbolines) and tryptamines related to serotonin," J. Med. Chem., 7: 135-141, (1964)
42. Y. Yamanaka, M.J. Walsh and V.E. Davis, "Salsolinol, an alkaloid derivative of dopamine formed in vitro during alcohol metabolism," Nature, 227: 1143-1144, (1970)
43. A. Brossi, A. Focella and S. Teitel: "Alkaloids in mammalian tissue I. Condensation of 1-DOPA and its two mono-O-methyl ethers with formaldehyde and acetaldehyde," Helv. Chim. Acta, 55: 15-21 (1972)
44. G. Cohen, "Tetrahydroisoquinoline alkaloids in the adrenal medulla after perfusion with 'blood concentrations' of  $^{14}\text{C}$ -acetaldehyde," Biochem. Pharmacol. 20: 1757-1761 (1971)
45. M.A. Collins and G. Cohen, "Isoquinoline alkaloid biosynthesis from adrenal catecholamines during  $^{14}\text{C}$ -methyl alcohol metabolism in rats," Fed. Proc. 29: 608 (1970)
46. G. Cohen and R. Barrett, "Fluorescence microscopy of catecholamine-derived tetrahydroisoquinoline alkaloids formed during methanol intoxication," Fed. Proc. 28: 288 (1969)
47. J.H. Robbins "Possible alkaloid formation in alcoholism and other diseases," Clin. Res., 16: 554 (1968)
48. P. Holtz, K. Stock and E. Westermann, "Formation of tetrahydropapaveroline from dopamine in vitro," Nature, 203: 656-658 (1964)
49. M. Sandler, S.B. Carter, K.R. Hunter and G.M. Stern, "Tetrahydroisoquinoline alkaloids: In vivo metabolites of L-DOPA in man," Nature, 241: 439-443 (1973)
50. R.M. Dajani and S.E. Saheb, "A further insight into the metabolism of certain B-carbolines," Ann. N.Y. Acad. Sci., 215: 120-123 (1973)
51. J.A. Rubenstein and M.A. Collins, "Tetrahydroisoquinolines derived from noradrenaline-acetaldehyde condensations: Pyrogallol-sensitive O-methylation in rat homogenates," Biochem. Pharmacol., 22: 2928-2931 (1973)
52. A.C. Collins, J.L. Cashaw and V.E. Davis, "Dopamine-derived tetrahydroisoquinoline alkaloids: Inhibitors of neuroamine metabolism," Biochem. Pharmacol., 22: 2337-2348 (1973).
53. M. Bigdeli, M.S. Thesis, Loyola University of Chicago (1972)

54. G. Cohen, C. Mytilineou and R. Barrett, "6-7-dihydroxytetrahydro-isoquinoline: Uptake and storage by peripheral sympathetic nerve of the rat," Sci., 175: 1269-1272 (1972)
55. R. Heikkila, G. Cohen and D. Dembiec, "Tetrahydroisoquinoline alkaloids: Uptake by rat brain homogenates and inhibition of catechol amine uptake," J.Pharm.Exp.Ther., 179: 250-258 (1971)
56. R.E. Greenberg and G. Cohen, "Tetrahydroisoquinoline-stimulated secretion from the adrenal medulla," Fed.proc., 31: 521 (1972)
57. G. Cohen, "Tetrahydroisoquinoline alkaloids: Uptake, storage and secretion by the adrenal medulla and by adrenergic nerves," Ann.N.Y. Acad. Sci., 215: 116-119 (1973)
58. A.M. Hjort, E.J. De Beer, J.S. Buck and L.O. Randall, "Relative Pharmacological effects of 1-methyl-3,4-dihydro-and 1-methyl-1,2,3,4-tetrahydroisoquinoline derivatives," J. Pharm. Exp. Ther., 76: 263-269 (1942)
59. A.M. Hjort, E.J. De Beer, and D.W. Fassett, "Some tetrahydroisoquinolines I. The relative toxicology and symptomatology," J. Pharm. Exp. Ther., 62: 165-173 (1938)
60. E. Toth, G. Fassina, and E.S. Soncen, "Cardiovascular and lipid mobilizing effects of tetrahydroisoquinoline," Arch. Intern.Pharmacodyn., 169: 375-383 (1967)
61. R.F. Shank, D.D. Miller and D.R. Feller, "Influence of substituted tetrahydroisoquinolines and catecholamines on lipolysis in vitro-II: Stereoselectivity" Biochem. Pharmacol., 20: 3403-3412 (1971)
62. A.T. Shulgin, "Mescaline: The chemistry and pharmacology of its analogs," Lloydia, 36: 46-58 (1973)
63. M.A. Collins, "Tetrahydroisoquinoline alkaloids from condensation of alcohol metabolites with norepinephrine: Preparation, synthesis and potential analysis in nervous tissue by gas chromatography," Ann. N. Y. Acad. Science, 215: 92-97 (1973)
64. S.F. Dyke and A.C. Ellis, "The synthesis of isopavine alkaloids-1," Tet., 27: 3803-3809 (1971)
65. P.P. Laidlow, "The action of tetrahydropapaveroline," J. Physiol., 40: 480-491 (1910)
66. P. Holtz, K. Stock and E.Westermann, "Pharmakologie des tetrahydropapaveroline und sein entstehung aus dopamin," Naunym-Schmiedeberg's Arch. Exp. Pathol. Pharmacol, 248: 387-405 (1964)
67. R. Santi, A. Brani, S. Luciani, C.E. Toth and A.R. Contessa, "Pharmacological properties of tetrahydropapaveroline and their relation to the catecholamines," J. Pharm. Pharmacol., 16: 287-288 (1964)

68. R. Santi, M. Ferrari, C.E. Toth, A.R. Contessa, G. Fassina, A. Bruni and S. Luciani, "Pharmacological properties of tetrahydropapaveroline," J. Pharm. Pharmacol., 19: 45-51 (1967)
69. S. Amit and M.H. Stern, "Electrochemical interaction of medical forebrain bundle and alcohol dependence in the laboratory rat," in Biological Aspect of Alcohol Consumption, (O. Forsander and K. Eriksson, eds.) pp. 225-231, The Finnish Foundation for Alcohol Studies, Helsinki (1972).
70. B. Kissin, V. Schenker and A. Schenker, "The acute effect of ethyl alcohol and chlorpromazine on certain physiological functions in alcoholics," Quart. J. Stud. Alc., 20: 480-492 (1959)
71. R.F. Docter and R.B. Perkins, "The effect of ethyl alcohol on autonomic and muscular response in humans. I. Dosage of 0.5 millilitre per kilogram," Quart J. Stud. Alc., 22: 374-386 (1961)
72. P.V. Halushka and P.C. Hoffman, "Alcohol addiction and tetrahydropapaveroline," Science, 169: 1104-1105 (1970)
73. V.E. Davis and M.J. Walsh: Science, 169: 1105-1106 (1970)
74. H. Sprince, C.M. Parker, G.G. Smith and L.J. Gonzales, "Alcoholism: Biochemical and nutritional aspects of brain amines, aldehydes and amino acids," Nat. Rep. Intern., 5: 185-200 (1972)
75. G. Freund, "Alcohol withdrawal syndrome in mice," Arch. Neurol., 21: 315-320 (1969)
76. F.W. Ellis and J.R. Pick, "Experimentally induced ethanol dependence in Rhesus monkey," J. Pharm. Exp. Ther., 175: 83-88 (1970)
77. E. Majchrowicz, "The concentration of ethanol and acetaldehyde in blood and brain of alcohol dependent rats," Trans. Amer. Soc. Neurochem., 4: 113 (1973)
78. W. A. Pieper, M. J. Skeen, H.M. McClure and P.G. Bourne, "The chimpanzee as an animal model for investigating alcoholism," Science, 176: 71-74 (1972)
79. D.B. Goldstein, "Quantitative study of alcohol withdrawal signs in mice," Ann. N.Y. Acad. Sci., 215: 218-223 (1973)
80. C.G. Hammar, Bo. Halmstadt, J.E. Lindgren and R. Tham, "The combination of gas chromatography and mass spectrometry in the identification of drugs and metabolites," Adv. Pharmacol. Chemother. 71: 53-89 (1969)
81. E. Leete, "The biogenesis of morphine," J. Am. Chem. Soc., 81: 3948-3951 (1959)

82. M. J. Horning, A.M. Moss and E.S. Horning, "A new method for separation of the catecholamines by gas-liquid chromatography," Biochem. Biophys. Acta, 148: 597-600 (1967)
83. E.L. Arnold and R. Ford, "Determination of catechol containing compounds in tissue samples by gas-liquid chromatography," Anal. Chem. 45: 85-89 (1973).
84. I.L. Martin and G.B. Ansell, "A sensitive gas chromatographic procedure for the estimation of noradrenaline, dopamine and 5-hydroxytryptamine in rat brain," Biochem. Pharmacol., 22: 521-533 (1973)
85. S.H. Koslow, F. Cattabeni and E. Costa, "Norepinephrine and dopamine: Assay by mass-fragmentography in the picomole range," Science, 176: 177-180 (1972)
86. S. Agurell, "Identification of alkaloid intermediates by gas chromatography-mass spectrometry. I. Potential mescaline precursors," Illoydia, 32: 40-45 (1969)
87. J. L. Cashaw, M.J. Walsh, Y. Yamanaka and V.E. Davis, "Simultaneous determination of biogenic amines and narcotic alkaloids by gas-liquid chromatography," J. Chrom. Science, 9: 98-104 (1971)
88. M.A. Collins and F.J. Kernozek, "Catecholamine-related isoquinoline alkaloids. I. Synthesis of 4-hydroxy-tetrahydroisoquinoline analogs of adrenaline and metanephrine," J. Heterocycl. Chem., 9: 1437-1440 (1972)
89. A.H. Anton and D.F. Sayre, "A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines," J.Pharm. Exp. Ther., 138: 360-375 (1962)
90. H. M. McNair and E.J. Bonelli, Basic Gas Chromatography, p. 64, Varian Aerography (1968)
91. K. Imai, N. Arizuni, M.T. Wang. S. Yoshive and Z. Tamura, "Studies on the metabolism and extraction of L-3,4-dihydroxyphenylalanine (L-DOPA) in human beings by gas chromatography," Chem. Pharm.Bull. (Tokyo), 20: 759-764 (1972)
92. B.B. Coldwell, G. Solomonraj, H.L. Trenholm and G.S. Wiberg, "The gas chromatographic estimation of ethanol, acetaldehyde and acetone in ethanol metabolism studies," Clin. Toxicol, 4: 99-113 (1971)
93. L.E. Scheving, W.H.Harrison, P.Gordon and J.E.Pauly, "Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain," Amer. J. Physiol., 214: 166-173 (1968)
94. D.D. Clarke, S. Wilk and S.E. Gitlow, "Gas chromatographic determination of dopamine and related compounds," Biochem. Applic. Gas Chrom. 2: 137-163 (1968)



95. K. Imai, M. Sigiura and Z. Tamura, "Catecholamines in rat tissue and serum-Determination by gas chromatography method," Chem. Pharm. Bull. (Tokyo), 19: 409-411, (1971)
96. J. Barchas, E. Erdelyi and P. Angwin, "Simultaneous determination of indole-and catecholamines in tissue using a weak cation exchange resin," Anal. Biochem. 50, 1-17 (1972)
97. M.K. Shelenberger and J.H. Gordon: "A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas." Anal. Biochem., 39: 356-372 (1971)
98. E. Majchrowicz and J.H. Mendelson, "Blood concentrations of acetaldehyde and ethanol in chronic alcoholics," Science, 168, 1100-1102 (1970)
99. S. Wilk and B. Zimmerberg, "Absence of 3-methoxy-4 hydroxyphenylethanol in brain," Biochem. Pharmacol., 22: 623-628 (1973)
100. U.S. Von Euler, U. Hamberg, and S. Hellner, "B-(3,4-dihydroxyphenylethylamine (hydroxytyramine) in normal human urine, Biochem. J., 49: 655-658 (1951)
101. D.D. Clarke, S. Wilk, S.E. Gitlow and M.J. Franklin, "Gas chromatographic determination of dopamine at the nanogram level," J. Gas Chrom., 5: 307-310 (1967)
102. U. S. Von Euler, Noradrenaline, Charles C. Thomas, Springfield, Ill. (1956)
103. G. Cohen, Columbia University, N.Y., N.Y., Personal communication
104. M.J. Walsh, V.E. Davis and Y. Yamanaka, "Tetrahydropapaveroline, an alkaloid metabolite of dopamine in vitro," J. Pharm. Exp. Ther., 144: 388-400 (1970)
105. M.A. Collins, R. Gordon, M.G. Bigdeli, and J.A. Rubenstein, "Pyrogallol-a potent elevator of acetaldehyde during ethanol metabolism," Chemico-Biol. Interactions, in press. Nov. 1973.
106. W.C. Butts, "Two column gas chromatography of trimethylsilyl derivatives of biochemically significant compounds," Anal. Biochem., 46: 187-199 (1972)
107. T.R. Tephly, R.E. Parks and G.J. Mannering, "Methanol metabolism in the rat," J. Pharm. Exp. Ther., 143, 292-300 (1964)
108. N.H. Raskin and L. Sokoloff: "Brain alcohol dehydrogenase." Science, 162, 131-132 (1968)
109. K. Blum, W. Calhoun, J.E. Wallace, J.H. Merrit and I. Geller, "Soporific action of ethanol in mice: possible role of biogenic amines," Pharmacol. Biochem. Behav. 1: 271-276 (1973)

110. H.C. Kim and A. D'Iorio, "Possible isoenzymes of monoamine oxidase in rat tissue," Can. J. Biochem., 46: 295-297 (1968)
111. J.P. Johnston, "Some observations upon a new inhibitor of monoamine oxidase in brain tissue," Biochem., Pharmacol., 17: 261-268 (1968)
112. M.B.H. Youdim, G.G.S. Collins, and M. Sandler, "Multiple forms of rat brain monoamine oxidase," Nature, 223: 626-628 (1969)
113. L. Sierens and A. D'Iorio, "Multiple monoamine oxidase in the rat liver mitochondria," Can. J. Biochem., 48: 659-663 (1970)
114. L.A. Romanova and V.Z. gorki, "Selective inhibition of biogenic amine oxidation with pargyline or phenelzine," Formakologiya i Toxicologiya, 32: 667-669 (1969)
115. E.A. Zeller, "A new approach to the analysis of the interactions between monoamine oxidase and its substrate and inhibitor," Ann. N.Y. Acad. Sci., 107: 811-820 (1963)
116. R.F. Squires, "Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: A comparison of eight mammalian species," Adv. Biochem. Psychopharm., 5: 355-370 (1972)
117. E.A. Zeller, Northwestern University, Chicago, Personal communication.
118. R. Pfeiffer and M. Ebadi: On the mechanism of the nullification of CNS effects of 1-DOPA by pyridoxine in Parkinsonian patients. J. Neurochem., 19: 2175-2181 (1972)
119. H. Corrodi and N.A. Hillarp, "100. Fluoreszenznitodin zur histochemischen sicht barmachung von monoaminen. 2. (1) Identifizierung des fluoreszierenden productes aus dopamine und formaldehyd," Helv. Chim. Acta, 47: 911-918 (1964)
120. P. Molinoff and J. Axelrod, "Biochemistry of CAs," in Ann. Rev. Biochem., 40: 465-500 (1971)
121. K.H. Graefe, F.J.E. Stefano, and S.Z. Langer, "Preferential metabolism of ( — )-<sup>3</sup>H-norepinephrine through the diaminated glycol in the rat was deferens," Biochem. Pharmacol., 22: 1147-1160 (1973)
122. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R. J. Randall, "Protein measurement with the Folinphenol reagent," J. Biol. Chem., 193: 265-275 (1951)
123. E. Anggard and G. Sedvall, "Gas chromatography of catecholamine metabolites using electron capture detector and mass spectrometry," Anal. Chem., 41: 1250-1256 (1969)

124. U.S. Von Euler, "Specific sympathomimetic ergone in adrenergic nerve fiber (sympathin) and its relation to adrenaline and noradrenaline," Acta Physiol. Scand., 12: 73-97 (1946)
125. L.M. Gunne, "Catecholamine metabolism and morphine abstinence," Ann. N.Y. Acad. Sci., 96: 205-210 (1962)
126. K.A. Montague, "Adrenaline and noradrenaline concentrations in rat tissue," Biochem. J. 63: 559-565 (1956)
127. I. Sano and K. Taniguchi, Klin. Wochs., 38: 57 (1960)
128. A. Carlsson, "The occurrence, distribution and physiological role of catecholamine in the nervous system," Pharmacol. Rev., 11: 490-493 (1959)
129. E.G. McGeer and P.L. McGeer, "Catecholamine content in spinal cord," J. Can. J. Biochem., 40: 1141-1151 (1962)
130. G. Cohen, "A role for tetrahydroisoquinoline alkaloids as false adrenergic neurotransmitters in alcoholism," Advances Exp. Med. Biol., 35: 33-35 (1973)
131. N.J. Uretsky and L.L. Iversen, "Effect of 6-hydroxydopamine on catecholamine containing neurons in the rat brain," J. Neurochem., 17: 269-278 (1970)
132. B. Tabakoff, F. Ungar and S.G.A. Alivisatos, "Addiction to barbiturate and ethanol: Possible biochemical mechanisms," Advances Exp. Med. Biol. 35: 45-55 (1973)
133. A. Saner and H. Toenen, "Model experiments on the molecular mechanism of 6-hydroxydopamine," Mol. Pharmacol., 7: 147-154 (1971)
134. J.W. Maas and D.H. Landis, "The technique for assaying the kinetics of norepinephrine metabolism in the central nervous system in vivo," Psychosom. med., 28: 247-256 (1966)
135. J. Friedhoff and J. Miller, "Effect of ethanol on biosynthesis of dopamine," Ann. N.Y. Acad. Sci., 215: 183-186 (1973)
136. H.M. Adam, "The topology of brain amines-A review," in Metabolism of Amines in Brain (G. Hooper ed.) pp. 1-9, Mc Millan, N.Y. (1968)
137. N.K. Mello, "A review of methods to induce alcohol addiction in animals," Pharmacol. Biochem. Behav., 1: 89-101 (1972)
138. D. Lester, "Criteria for an animal model of alcoholism," Pharmacol. Biochem. Behav., 1: 103-107 (1972)
139. S.M. Schanberg, J.J. Schildkraut, J.R. Breese and I.J. Kopin, "Metabolism of normetanephrine-<sup>3</sup>H in rat brain- identification of conjugated 3-methoxy-4-hydroxyphenylglycol as the major metabolite," Biochem. Pharmacol., 17: 247-254 (1968)

140. J. Axelrod, "The metabolism, storage and release of catecholamines,"  
Recent Progr. Hormone research, 21: 597-622 (1965)
141. J.P. Hanig, J.M. Morisson Jr., and S. Krop, "Ethanol enhancement  
of blood-brain barrier permeability to catecholamines in chicks,"  
Europ. J. Pharmacol., 18: 79-82 (1972)

## APPENDIX I

AcD	acetaldehyde
CA	catecholamine
CNS	Central nervous system
COMT	catechol O-methyltransferase
DA	dopamine
DHPG	3,4-dihydroxyphenylglycol
DOPA	3,4-dihydroxyphenylalanine
EC/GC	electron capture gas chromatography
E	epinephrine
GC	gas chromatography
HFB-	heptafluorobutyric-
HCHO	formaldehyde
5-HIAA	5- hydroxyindole acetic acid
5-HT	5- hydroxytryptamine
i.p.	intraperitoneal
i.v.	intravenous
LRT	long retention time
MAO	monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenyl glycol
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NE	norepinephrine
PFP-	pentafluoropropionic-
TFA-	trifluoroacetic-
THP	tetrahydropapaveroline

Appendix I (continued)

TIQ	1,2,3,4-tetrahydroisoquinoline
VMA	vanillylmandilic acid

# APPENDIX II

## EFFECT OF ETHANOL ON ADRENAL GLAND PROTEIN AND CA CONCENTRATIONS

Drug <sup>1</sup> Treatment	Weight of adrenal gland (mg)	Protein % Conc. change mg/gland prot. from C		CA (ug/g tissue)			CA (ug/g protein)		
				DA	NE	E	DA	NE	E
Pyrogallol/ saline (6) (C)	25.7 ± 1.0	2.45 ± 0.09	-	9.7 ± 2.0	168 ± 35	434 ± 41	103 ± 21	1768 ± 180	4557 ± 330
Pyrogallol/ ethanol (6)	31.2 ± 1.4*	2.65 ± 0.12**	10.5	12.5 ± 3.2**	138 ± 19**	224 ± 39*	132 ± 22*	1315 ± 188**	2403 ± 410*

1 number of rats/group in parenthesis

\* p < 0.01 compared to the pyrogallol/saline control

\*\* p < 0.05 compared to the pyrogallol/saline control

### APPROVAL SHEET

The dissertation submitted by Mostafa G. Bigdeli has been read and approved by a committee from the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference content, form and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

January 11, 1974  
Date

Michael A. Collins  
Signature of Advisor